

EXHIBIT L

IN THE UNITED STATES DISTRICT COURT
FOR THE EASTERN DISTRICT OF WASHINGTON

IN RE:

CITY OF SPOKANE, a municipal corporation
located in the County of Spokane, State of
Washington, v. MONSANTO COMPANY,
et al.

Case No. 2:15-CV-00201-SMJ

EXPERT REPORT OF
DAVID L. EATON, PH.D., DABT, FATS

A handwritten signature in black ink, appearing to read "David L. Eaton", followed by a horizontal line.

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November 15, 2019

Expert Report of David L. Eaton

City of Spokane v. Monsanto

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Expert Report of David L. Eaton***City of Spokane v. Monsanto*****I. Introduction**

My name is David L. Eaton. I currently hold the title of Professor Emeritus of Environmental and Occupational Health Sciences, School of Public Health, and Dean Emeritus of the Graduate School, University of Washington, Seattle, WA. I also hold an appointment as Adjunct Professor of Pharmacology and Toxicology at the University of Arizona College of Pharmacy, Tucson, AZ. I received my Ph.D. in Pharmacology and Toxicology from the University of Kansas Medical Center in 1978. I was recruited soon after to the University of Washington faculty in the School of Public Health, Department of Environmental and Occupational Health Sciences, where I have spent the past 40 years of my career. During those 40 years, I taught classes in toxicology, conducted research on how chemicals cause cancer, served in a number of research administration roles. I maintained an active research program, receiving over \$60 million in federal grant dollars for my research. I have published 126 original peer-reviewed research articles, and 60 book chapters and education articles, and edited three books, all in the area of toxicology. Among these book chapters is the Principles of Toxicology chapter in the past 6 editions of *Casarett and Doull's Toxicology: The Basic Science of Poisons*, considered the leading textbook in the world in the field of toxicology. I served as founding Director of the National Institutes of Environmental Health Sciences Core Center of Excellence at the University of Washington for nearly 20 years. I also served as the Director of the NIEHS Superfund Research Program for 10 years. In the School of Public Health, I served for 8 years as the Associate Dean for Research before being asked by the UW Provost and President to serve as Associate Vice Provost for Research for the entire University of Washington. I served in that capacity for 8 years, while maintaining my research and teaching activities. I served for one year as Interim Vice Provost for Research, before being selected as Dean of the UW Graduate School and Vice Provost for Graduate Education for all three UW Campuses. I served in that role for the 5 years previous to my retirement from the UW in 2019. Professionally, I have held numerous leadership positions in the field of toxicology, including serving as Secretary, then Vice President and President of the Society of Toxicology, the largest international professional organization of toxicologists in the world, with over 8,000 members. I became Board Certified in Toxicology by the American Board of Toxicology in 1981, and served on the Board of Directors and as Treasurer of the American Board of Toxicology from 1991-94. I served on the Advisory Council of the National Institute of Environmental Health Sciences (NIEHS) from 2013-17. NIEHS is the branch of the National Institutes of Health that promotes and funds the vast majority of research on the toxic effects of chemicals found in our environment. I served for 8 years as the Chair of the Research Committee for the Health Effects Institute (HEI), a non-profit public-private partnership organization that funds research on the health effects of automobile-related air pollution. I have also provided extensive service to the National Academies of Science, Engineering and Medicine (NASEM; formerly called the National Academies of Science, NAS, and the Institute of Medicine, IOM) through service on over a dozen NASEM Report Committees on environmental hazards. For example, I served on the NAS/NRC Committee to Review the Drinking Water Standard for Arsenic in 2001. In 2004-06 I served as Chair of the NAS Committee on Assessment of the Health Implications of Exposure to Dioxin and Dioxin-Like Compounds, and in 2011-12 I served on the NAS/NRC Committee on the Future of Science at the EPA, and in 2012-14 I served on the NAS/NRC Committee to

Review the EPA IRIS process. More recently, in 2017-18, I served as Chair of the NASEM Review of the Public Health Consequences of e-Cigarettes. In 2018 I also served as Chair of the NIEHS Scientific Review Panel for the National Toxicology Program (NTP) Study on the 2-year rat Bioassay evaluating the potential cancer-causing effects of cell phone radiation. Over my career I've been fortunate to have received a number of honors and awards for my contributions to the discipline of toxicology, including:

- Fellow (elected), American Association for the Advancement of Science, 1995
- Fellow, International Union Against Cancer, 1996
- Fellow (Elected), Academy of Toxicological Sciences, 2000
- National Associate, National Academy of Sciences, selected 2004 (lifetime membership)
- Elected Fellow, Washington Academy of Sciences, 2011
- Elected Member, Institute of Medicine (now National Academy of Medicine), National Academies of Science Engineering and Medicine, 2011
- Public Communications Award, Society of Toxicology, 2014
- Excellence in Pharmacology/Toxicology Award, PhRMA Foundation, 2015

Although I retired from the University of Washington in 2019, I remain professionally active. I currently serve on the Board of Scientific Councilors of the National Toxicology Program, which is a Program within the NIEHS that is congressionally mandated to conduct research on chemicals found in the environmental, with special emphasis on determining which chemicals pose a cancer risk to humans. The NTP is responsible for evaluating the cancer-causing potential of chemicals and publishes the annual 'Report on Carcinogens.' I am also on the Board of Trustees of the Health and Environmental Sciences Institute (HESI), and international public-private partnership research organization. I also spend a small amount of my time as a consultant in toxicology. A copy of my CV is included in Appendix 1. I am compensated for my work in this matter at \$600 per hour.

A. Charge

I was retained by lawyers representing Monsanto as a testifying expert witness in the field of toxicology, and was asked to:

1. Determine whether the levels of PCBs in edible fish in the Spokane River System make them unsafe for human consumption.
2. Evaluate the state of the science of toxicology for industrial chemicals from the 1930's to the 1970's, and whether Monsanto's practices were consistent with standard toxicological practices of the time and whether Monsanto's warnings were consistent with toxicological knowledge in the corresponding timeframes.
3. Specifically evaluate the state of toxicological testing for cancer in that time period (1930's on) and whether there were any statutory, regulatory or industrial standards that would have required Monsanto to conduct animal testing for cancer.

4. Determine if an animal test for cancer performed in the 1930's - 60's would have demonstrated that PCBs cause cancer in laboratory animals.
5. Review and comment on plaintiffs' toxicology experts' reports.

B. Summary of Major Opinions:

1. The levels of PCBs measured in edible fish in the Spokane River do not make them unsafe for human consumption

A standard way of assessing the safety of potentially toxic substances in food is to conduct a 'Margin of Exposure' analysis. This requires that one establish a reliable estimate of daily intake (dose, in mg/kg/day) of the substance and compare that exposure to a measured dose in laboratory animals that has no observable adverse effect. This ratio of non-toxic dose in experimental animals to a human exposed dose is referred to as a "margin of exposure" (MOE). MOEs estimated using reasonable upper bound estimates of the amount of PCBs that could be consumed in fish from the Spokane River, based on PCB concentrations in fish and fish consumption data from the Spokane River and data for various toxicological endpoints, all exceed a value of 100, and in nearly all instances exceed 1,000. A MOE analysis using PCB concentration and fish consumption data from the Spokane River for various toxicological endpoints all exceed a value of 100, and in nearly all instances exceed 1,000. This means that, even for people consuming relatively large amounts of fish from the Spokane River, the dose of PCBs they receive is at least 1,000 times less than the dose given to laboratory animals that had no evident toxic effect and therefore is highly unlikely to have any biologically significant adverse effect, demonstrating that the PCB levels found in fish from the Spokane River do not make them unsafe for human consumption.

For cancer and immunotoxic effects, which have been used in the past by EPA to set acceptable daily intake levels for PCBs, MOEs for reasonable upper bound exposures for consumers were more than 100,000 for cancer and more than 27,000 for 8 different immunological endpoints studied in laboratory animals.

Based on MOEs that are well above 100 for all non-cancer end points, it is my opinion that the PCBs found in fish in the Spokane River at the levels measured, do not make them unsafe for human consumption.

The assessment and conclusions presented here are reinforced by the US EPA. Comparison of predicted exposure levels to US EPA reference doses (RfDs) for Aroclors (which are mixtures of different PCB congeners) suggests that no adverse noncancer effects are expected. An RfD is defined as an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily oral exposure for a chronic duration (up to a lifetime) to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime (US EPA, 2019d). US EPA has set oral RfDs for noncancer endpoints for Aroclor 1016 and Aroclor 1254 of 0.07 and 0.02 µg/kg-d, respectively. The RfD for Aroclor 1016 is based on reduced birth weights in monkeys and the RfD for Aroclor 1254 is based on immune, dermal and ocular effects in monkeys (US EPA, 1993, 1994). As explained later in this report, these RfDs are derived by applying safety factors (some of which are inapplicable) and are intended to be hundreds of times lower than the dose of PCBs observed to cause adverse effect in animal studies. My assessment estimates a hypothetical extreme and impossible daily dose of total PCBs by Spokane River fish consumers of 0.024 µg/kg-d if one

takes the upper 95th percentile intake rate AND the upper 95th percentile PCB uncooked fillet concentration.¹ It is impossible a consumer would be in BOTH the upper 95% for consumption rate AND have ALL of the fish they consume be in the upper 95% of PCB concentration over a lifetime of consumption. A much more reasonable, but still conservative, upper bound intake rate comparison to the EPA RfD would be to use the mean consumption rate and upper 95% concentration, or the upper 95% consumption rate and mean concentration, which correspond to total PCB intake rates of 0.005 and 0.014 µg/kg-d, respectively, and are well below the outdated but still used EPA RfDs for noncancer risk. Thus, comparison of estimated upper bound intake rates from consuming Spokane River fish to the EPA RfDs demonstrates that PCBs found in fish in the Spokane River are well below even the older, conservative 'safe dose' estimates of the EPA, and thus are highly unlikely to cause any type of noncancer effects in humans.

As a public health professional, it is important to consider the magnitude of public health benefit gained when an action is taken to 'reduce a potential risk.' In the circumstances of efforts to reduce PCBs in the Spokane River, if the current levels of PCBs in fish do not represent any significant potential risk to consumers (they do not), then the efforts to reduce this level will have a net public health gain of 0, and at an unnecessary expense. Indeed, given the potential to discourage consumption of a healthy food source (fish), it is very possible that this project would result in a net **negative** public health benefit to the citizens of Spokane and surrounding communities.

2. Monsanto's use of toxicological expertise and studies in the 1930's-60s was consistent with industry standards and practices of the time

Monsanto relied upon the top public health experts of the time to establish safe exposure levels in the workplace in the 1930's-60's, and commissioned hundreds of standard toxicology tests used to evaluate toxicity of industrial chemicals that were not intended for use as pharmaceuticals, food additives or pesticides used on food crops.

3. Consistent with the industrial toxicology practices of the time, Monsanto had no reason to conduct cancer studies in laboratory animals in the 1930's-60's because:

- a) PCBs were never intended for use as pharmaceuticals, food additives, or as pesticides used on food crops;
- b) There was never any evidence that occupational exposures to PCBs caused cancer in workers exposed to high levels;
- c) There was nothing about the chemical structure of PCBs that would have suggested it might be carcinogenic, based on chemical knowledge at the time;
- d) None of the early studies on the toxicology of PCBs, including Dr. Drinker's studies, provided any compelling evidence that would suggest PCBs might be carcinogenic, and none of the experts Monsanto relied upon ever suggested that PCBs should be tested for carcinogenic activity.

¹ The combined 95th percentile for both fish consumption rate AND 95th percentile for fish tissue concentration is a hypothetical/impossible scenario for a consumer because it corresponds to (1) a lifetime of consuming fish at extremely high intake rates, (2) a lifetime of daily consumption of only fish with the highest PCB levels, and (3) concentrations associated with raw fish tissues (because no correction factor was included to account for loss of about 30% of PCBs that occurs with cooking fish).

There were no statutory or regulatory requirements for testing industrial chemicals for cancer during the time that PCBs were manufactured by Monsanto. Furthermore, there was no evidence from early toxicology studies with PCBs that would have led a reasonable scientist of the time to suspect that PCBs would cause cancer. Indeed, none of the toxicology experts relied upon by Monsanto, or other scientists who conducted toxicity testing on PCBs, ever recommended to Monsanto that they conduct a cancer test in laboratory animals.

4. Based on my review of Monsanto's product warnings, and the state of knowledge of PCBs toxicology at the time, it is my opinion that the product labels and warnings were adequate, and consistent with the knowledge at the time

PCBs being manufactured in the 30's, 40's and 50's were industrial chemicals primarily used in 'closed' systems, but were used in other applications as well. The recognized hazards at the time related to inhalation exposure to vapors during manufacturing, and possible skin contact in certain occupations that used Aroclors in transformers, capacitors, hydraulic fluids, etc. Based on toxicological information available at the time, instructions to 'use adequate ventilation', avoid skin and eye contact, and similar product warnings were appropriate (e.g., Monsanto Chemical Company, 1943, 1947a, b, 1954, 1955, 1957, 1958, 1960, 1968a, b, 1969; 1970; Attachment 1C).

5. It is highly unlikely that a cancer study done in laboratory animals in the 1930's-60's would have found an excess of cancers from PCBs

The development of standardized carcinogen testing protocols was not completed until the National Cancer Institute established such protocols in the 1960's and published them for the first time in 1976 (National Cancer Institute, 1976). An evaluation of all known 'studies' in experimental animals identified in the Hartwell Compendium in 1941 demonstrates that there was no consistent approach to carcinogenesis studies and that studies being undertaken during this period of time used primitive methods compared to cancer studies conducted in the 1970's and later. Furthermore, of the 27 PCB cancer bioassays completed on rats and mice from 1971-1998, only 7 had a statistically significant increase in cancers. Of those 7, the only consistently positive outcome was for a single Aroclor among all those commercially available. Had Monsanto conducted such a test using techniques available at the time, it is likely that they would have chosen inhalation or dermal application as the route of exposure, as those routes of exposure were the only significant human pathways of exposure known or expected to occur. It is extraordinarily unlikely that either route of exposure would have resulted in malignant tumors (cancers), as the dose delivered to the liver, the primary site of tumors seen in rats using the oral route of administration, would have been well below that needed to induce malignant tumors in the liver. Further, it is highly unlikely that they would have chosen lifetime studies (2 years in rodents), as these were seldom done for industrial chemicals. Finally, it is highly unlikely that they would have used a sufficient number of animals to see a significant increase in malignant tumors. Any one of these factors makes it unlikely that a cancer test done prior to the 1960's would have been 'positive.'

6. Plaintiffs' experts in toxicology failed to use current methodologies and approaches to address potential health risks of PCBs in consumers of fish harvested from the Spokane River

Plaintiffs' experts DeGrandchamp and Olson failed to address the two key aspects of toxicology -- dose and dose-response for various toxicological outcomes -- and failed to use any accepted methodologies to

synthesize the data necessary to reach reliable conclusions. Without these key steps, a toxicologist cannot demonstrate causal associations between adverse health effects seen in animal experiments administered high doses with the fleetingly small doses of PCBs that occur from consumption of fish from the Spokane River. Their reports focused solely on 'hazard.' In toxicology, hazard is a specific term of art used to denote possible health endpoints absent of dose. There is no effort to address risk (the probability that the hazard becomes 'real' under a given set of circumstances, the most important of which is **human dose**). In the context of public health decision-making, hazard by itself is meaningless, and provides little useful information on which to make important public health decisions. Thus, their reports are misleading and provide no useful context from which to assess the actual level of human health risks, if any, of PCBs found in fish in the Spokane River.

Conclusions offered by plaintiffs' experts that Monsanto should have conducted cancer studies in the 1930s-1960s are not based on sound scientific principles or a correct interpretation of the state of the science at that time in history. Plaintiffs' experts' conclusions that, had Monsanto conducted a cancer bioassay prior to the late 1960s, it would have demonstrated PCBs to be carcinogenic is also not consistent with extensive evidence that strongly suggests that it is highly likely that such a study performed at that time would not have found statistically significant increase in cancers.

To the extent additional information becomes available, I may modify or add to the above opinions.

C. Introduction to the principles of toxicology

The role of a toxicologist in the courtroom is to help the judge and jury understand the fundamental concepts of toxicology as they apply to both the ‘potential to cause harm’ (hazard), and the ‘probability that harm has and/or will occur’ (risk), following alleged exposure to a hazardous substance. Although the ultimate goal in most toxic tort litigation is to elucidate the likelihood that exposure to a substance is capable of both causing and, in a particular case, did cause a disease or other adverse outcome in a particular individual, the science of toxicology is also used extensively in the regulatory arena, such as in the setting of emissions standards to protect air and water resources and the public health (EPA jurisdiction), to determine allowable concentrations of potentially toxic substances in foods (FDA jurisdiction), evaluate the safety and conditions for marketing of drugs (FDA jurisdiction) and the protection of consumers from potentially harmful substances introduced into the marketplace (CPSC).

But regardless of the specific tort claim surrounding exposure (individual or population) to a potentially toxic substance or mixture of substances, the fundamental principles of toxicology remain the same. I have written extensively on this topic, including providing ‘Principles of Toxicology’ chapters for the major textbooks in the field (Aleksunes and Eaton, 2019; Eaton, 1985; Eaton, 2018; Eaton *et al.*, 1998; Eaton and Gallagher, 1997, 2010; Eaton and Gilbert, 2008, 2013; Eaton and Hill, 1996; Eaton and Klaassen, 2001, 2003; Eaton and Robertson, 1994; Eaton and Schaupp, 2015; Eaton *et al.*, 2018; Gilbert and Eaton, 2009). In addition to writing for students and professionals new to the field, I’ve written two articles about the proper use of toxicology in the courtroom (Eaton, 2003; Eaton and Kalman, 1994), including one specifically for judges and lawyers (Eaton, 2003) at the request of American Association for the Advancement of Science and the Brooklyn Law School.

1. Basic concepts of the ‘Dose-Response Relationship’ and its importance to toxicology

A long-held paradigm in toxicology was first presented in the mid-17th century by the physician/scientist/philosopher, Paracelsus, who stated *“All substances are poisons; there is none which is not. The dose differentiates a poison from a remedy.”* Although gains in scientific understanding of how chemicals foreign to the body (xenobiotics) -including those both natural and man-made- are handled by the body has increased dramatically in the past 50+ years, this fundamental point has not changed. Indeed, much of regulatory toxicology is focused on identifying the highest dose which will have no adverse effect, often called the “No Observable Adverse Effect Level (NOAEL)” in experimental animal studies. In more recent years, the EPA and other regulatory agencies now use the terms ‘point of departure’ or ‘benchmark dose’ to calculate a ‘Reference Dose’ (sometimes referred to as a ‘virtually safe dose’, in humans), but these terms all represent conceptually the same idea – a representation of the highest dose that can be given repeatedly to experimental animals with no evidence of an adverse or toxic response. The term ‘dose’ refers to the amount of a substance potentially entering the body. But even this term is nuanced; depending on the nature of the exposure, the ‘exposed dose’ and the ‘biologically relevant dose’ may be the same, or drastically different. The ‘route’ of exposure can make a big difference in the likelihood of adverse outcome following an exposure to a toxic substance. There are 3 common routes of exposure: oral (usually ingestion); inhalation (breathing in vapors or air-borne particles) and dermal (direct contact with the skin). The fraction of the ‘exposed dose’ that is actually absorbed into the body is referred to as the ‘bioavailable’ dose, and this is what is most relevant toxicologically.

For substances that enter the body through oral ingestion, such as dietary contaminants like PCBs, the extent of absorption (bioavailability) is dependent on numerous factors, including stability of the substance in the presence of stomach acids and enzymes, fat solubility, and binding to other substances in the gastrointestinal (GI) track. PCBs are highly lipid soluble, and this favors absorption across the membranes in the intestinal track. Thus, it is generally assumed that the majority of the exposed dose (the amount of PCBs present in the diet) is absorbed into the body. An important point to recognize is that nearly all of the dose that is absorbed from the GI track goes directly to the liver before being distributed to the rest of the body. This is important because the liver is capable of either activating (making more toxic) or detoxifying and eliminating most toxic substances. Indeed, the liver can be viewed as a sort of 'in-line' metabolic filter, designed to prevent unwanted substances from entering the main bloodstream and being delivered to the rest of the body. But in many circumstances the liver is actually the site of toxicity (so-called 'target organ') because it receives a much higher dose, and has metabolic machinery that can, in the process of trying to eliminate it, make a substance more toxic rather than less. In experimental animals, the liver is the primary target organ for toxicity of PCBs when exposure is oral. In humans, exposure of the skin to high levels of some PCB mixtures can cause direct effects on the skin, as was occasionally seen in the workplace environment where PCBs were used extensively.

A second important aspect of 'dose' is the frequency and duration of exposure. Typically, public health scientists are not generally concerned with single, low dose exposures to a potentially toxic substance. Rather, it is repeated, frequent exposures over long periods of time (usually measured in years) to seemingly small amounts of chemical that often create the regulatory dilemma as to 'how much is safe' and 'how safe is 'safe enough'.' For substances that are quickly eliminated from the body, such as many medications like aspirin or acetaminophen (Tylenol), repeated exposures generally do not result in long-term accumulation in the body, because these substances have short biological half-lives (the time it takes to eliminate 50% of the substance from the body), typically measured in hours. In contrast, some substances, including most PCBs, have very long half-lives, usually measured in years. For such persistent organic chemicals, it is long-term (chronic) health effects, such as cancer, heart disease or organ system toxicity, that is often the focus of regulatory concern.

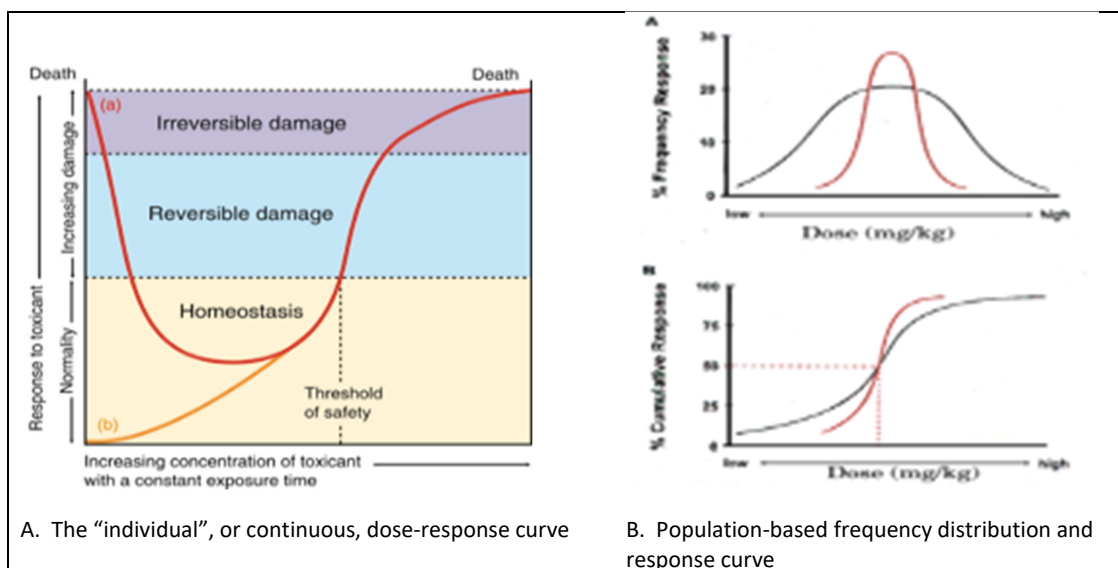


Figure 1. Dose-response relationships

Toxicologists recognize two fundamentally different types of 'dose-response relationship'. The first is the individual response – e.g., in an individual, the response seen will vary with the dose, such that there will be small doses with no effect, larger doses that have some effect such as nausea or vomiting, and at higher doses still perhaps more serious, even life-threatening effects such as heart attack, coma and death. The individual, or 'graded' dose response curve is shown in Figure 1A (Eaton and Klaassen, 2003). For certain substances, such as essential trace metals and vitamins, having too small of a dose can actually be toxic (e.g., a vitamin deficiency), illustrated by the ascending line on the left hand of Figure 1A. It is evident from Figure 1A that there are doses below which the toxic substance has no evident toxic effect, the so-called 'threshold of safety' in Figure 1A. However, individuals within a population will have some variability in their susceptibility to a toxic substance, such that the 'threshold dose' will actually be a distribution of doses in a population.

Thus, there is a second type of 'dose-response relationship' that is most frequently used in the regulatory arena, which is based on the frequency distribution of individual 'threshold' doses (i.e., at any given dose, each individual in the population either has a response, or does not have a response- referred to as a 'quantal' response). This is illustrated in Figure 1B, where the upper panel shows the frequency distribution of doses that cause a response in individuals in a population. When this distribution is plotted as a cumulative, quantal response, one obtains a 'population dose-response curve' (Figure 1B, lower panel). How scientists use this type of dose-response information to assess risks to the public health will be discussed later, after a brief introduction to how chemicals in the environment and diet can cause toxic effects in individuals and populations.

2. How chemicals like PCBs might cause adverse health effects in individuals and populations

There is a myriad of ways that chemicals- whether they are drugs, naturally occurring substances in the diet or synthetic chemicals found in the environment, can cause unwanted effects. Among the many potential 'modes of action', there are two broad categories that are highly relevant to PCBs: one is through activation of receptors, and the other is through metabolism in the body to so-called 'reactive intermediates' that can cause 'oxidative stress' and/or bind to DNA and cause genetic damage.

3. Basic concepts of 'receptor-ligand' interactions

Because much of the toxicity attributed to PCBs acts via a very specific mechanism, it is important to have a basic understanding of what pharmacologists and toxicologists refer to as 'receptor-ligand interactions.' Indeed, nearly all chemicals that can have a beneficial effect on the human body (the purview of the discipline of pharmacology-the study of how chemicals used as drugs/ pharmaceuticals act in the body) and many chemicals that have only unwanted, adverse effects (the discipline of toxicology) require a detailed understanding of this process.

A very large percentage of the 23,000+ genes in the human genome have the 'blueprint' for making proteins that have very important, and very specific effects. These proteins, called 'receptors' work through highly complex and integrated network of chemical and electrical signals. Receptors can be visualized as a lock that can remain in the 'off' position, until another molecule, either endogenous (produced in the body) or exogenous (environmental or dietary), interacts in a very specific way with the receptor (Figure 2). These molecules, referred to as 'ligands', bind in highly specific ways. There are two possible outcomes when a ligand interacts with its specific (sometimes called 'cognate') receptor – it can either turn the receptor to the 'on' position, or it can bind to and block the receptor without turning it on. If a ligand acts via the first process – switching the receptor to the 'on' position, the ligand is referred to as an 'activating ligand', or more properly as an 'agonist.' For ligands that bind to their cognate receptor, but don't activate it, they are referred to as 'antagonists', because they bind to - but don't activate - the receptor AND they prevent the endogenous ligand from binding to and activating that receptor.

A good example of the importance of this type of interaction is when the endogenous molecule, histamine, is released from certain cells in the body in response to another environmental signal, such as pollen. When the histamine is released, it binds very specifically to a family of receptors called, appropriately, histamine receptors, of which there are several (H1, H2, H3, etc.). Drugs called 'antihistamines' are antagonist ligands to H1, H2 and/or H3 receptors, blocking the histamine from binding to the receptor. Antihistamines represent a very useful class of molecules in the treatment of all kinds of allergies, and indeed can be lifesaving.

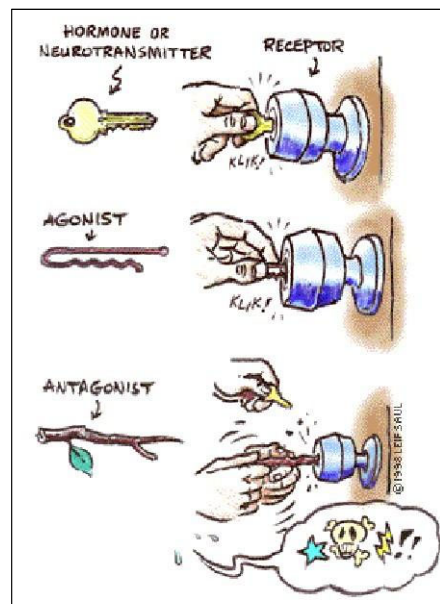


Figure 2. Conceptual model of ligand receptor interactions

Hormones, such as estrogen, testosterone, glucocorticoids, and thyroid hormone, are another example of activating ligands to specific receptors. Estrogen binds to the estrogen receptor (actually several slightly different types), testosterone binds to the testosterone receptor, and thyroid hormone binds to the thyroid hormone receptor. Exogenous chemicals can sometimes interact with these receptors. For example, the molecule diethylstilbestrol, or DES, is very potent agonist of the estrogen receptor, disrupting normal estrogen signaling. Women who took DES during pregnancy sometimes ended up with serious problems with their pregnancies, and their daughters who were exposed in the uterus have a higher rate of certain types of rare cancers.

Another example of 'environmental estrogen' exposure occurs from a wide range of plant-derived chemicals called 'phytoestrogens' (Shanle and Xu, 2011). Some of these chemicals, such as genestein, are potent estrogen agonists (act like estrogen) and occur at relatively high concentrations in soy and certain other plants. Other environmental pollutants, such as breakdown products of the pesticides, DDT and methoxychlor, also act via mimicking estrogen (i.e. they are estrogen receptor agonists). When an activating ligand (agonist) like estrogen or testosterone binds to its specific receptor, it triggers a series of actions, called 'downstream events', that can sometimes lead to significant changes in normal biology (Figure 3).

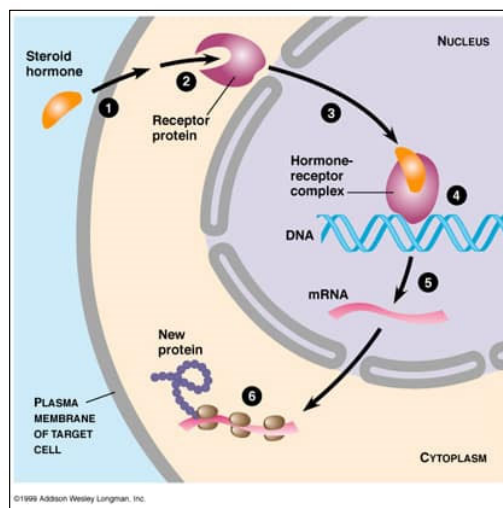


Figure 3. Hormone-receptor interaction leads to changes in gene expression and protein synthesis

In a similar manner, some chemicals can act as blocking ligands (antagonists). For example, the drug, tamoxifen, which is widely used in the treatment of estrogen-responsive breast cancer, is useful in treating breast cancer because it blocks the interaction of estrogen with the estrogen receptor, which is required for the growth of breast cancer cells. Chemicals that produce biological effects by interfering with hormone signaling pathways are frequently called 'endocrine disrupting chemicals' (EDCs), or simply 'endocrine disruptors.'

Both agonist and antagonist ligands can interfere with normal endocrine signaling. When the lock is opened (i.e., the agonist binds to and activates the receptor) a series of downstream events is initiated. These events usually involve changes in the way that other genes in a cell are turned on or off, a process called 'transcriptional regulation', because the information in the gene is transcribed into messenger RNA, which in turn is used to make a functional protein (referred to as 'translation') (Figure 3).

EDCs have two possible ways of interfering with normal endocrine signaling – one is referred to as a 'direct' effect, in which the molecule interacts directly with the endocrine receptor, either as an agonist, or an antagonist. But some chemicals, including some PCBs, can also modify endocrine pathways in an 'indirect' way. Generally, indirect EDCs act via changing the way hormones in the body are broken down (metabolized). These two broad mechanisms of action are illustrated in Figure 4. It is in this second way that PCBs have been classified as 'endocrine disruptors.' Exposure of animals to high doses of PCBs can lead to an

increase in the expression (i.e., turns on the genes, referred to as 'enzyme induction') of several enzymes in the body that are responsible for breaking down estrogen and other hormones.

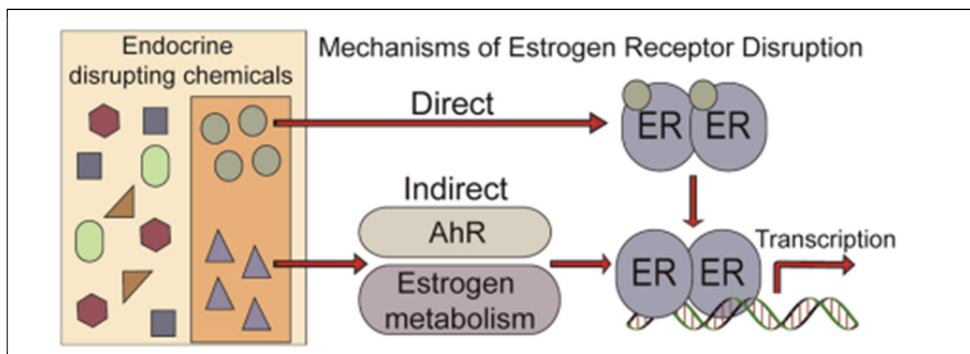


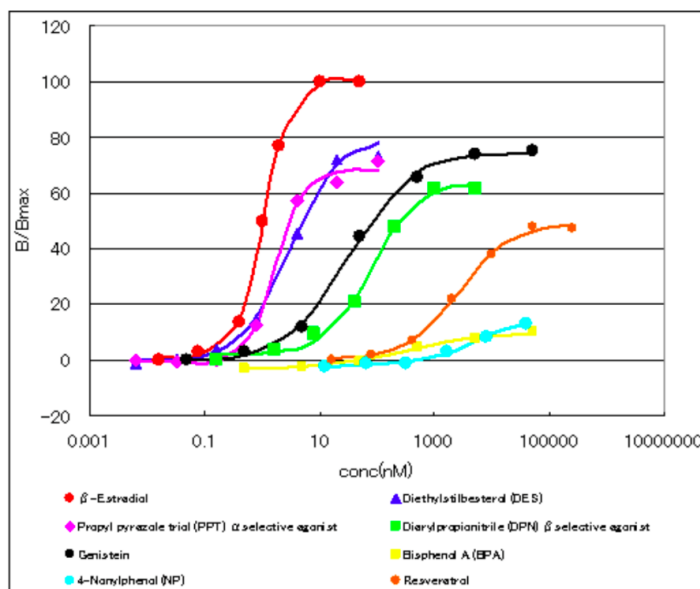
Figure 4. Direct and indirect mechanisms of endocrine disruption by exogenous chemicals (Shanle and Xu, 2011)

The evidence that PCBs can interact directly with hormone receptors is limited. However, some PCB molecules interact with other receptors in the body, which can dramatically change the way that certain hormones are metabolized. Of the 209 possible 'congeners' of PCBs, a small subset of 12 have been designated as 'dioxin-like' because they have the ability to interact with a very specific receptor called the 'Aryl hydrocarbon Receptor', or AhR (sometimes called the 'dioxin receptor'). Understanding how dioxin-like PCBs (DL-PCBs) bind to and activate the AhR is critical to the safety evaluation of PCBs, because much of the toxic effects of PCBs seen in laboratory animal studies are due to this very specific interaction. Note that "dioxin-like" does not imply that DL-PCBs contain any actual dioxin. Appendix 3 provides a detailed discussion of the AhR and its importance to understanding the toxicology of PCBs and the human health implications from environmental exposures to PCBs. Although the interaction of DL-PCBs with the AhR explains much of the observed toxicity of PCB mixtures in laboratory animals, there are also toxic effects mediated by non-DL-PCBs. However, much of the toxicity of these non-DL-PCBs, such as PCB153 (one of the most prevalent PCB congeners found in the environment), is still mediated by interactions with other receptors, such as the 'Constitutive Androstane Receptor' (CAR) and the 'Pregnane X-Receptor' (PXR). Some of the most prevalent non-DL-PCBs found in the environment, such as PCB138, 153 and 180, are all effective agonists (activate) of either rat PXR and/or CAR (Gahrs *et al.*, 2013), and this is likely to be a major 'mode of action' for non-DL-PCBs to act as cancer promoters in rats. However, as with the AhR, there appears to be important species differences between humans and rats for PCB-mediated activation of PXR and CAR. Tabb *et al.* (2004) demonstrated that human CAR and PXR respond completely differently than the rat CAR and PXR to PCBs 138, 153 and 180—the human CAR and PXR receptors are either not activated, or are inhibited, by these non-DL-PCBs (Tabb *et al.*, 2004). An international panel of experts convened to evaluate the relevance of rat liver tumors that occur following CAR activation concluded that this 'mode of action' of liver tumor formation in rats is not relevant to humans (Elcombe *et al.*, 2014). These studies, and their implications to extrapolating laboratory animal data to humans, are discussed in more detail in Appendix 3.

Another very important basic principal of receptor-ligand interactions needs to be discussed that brings us back to the concept of dose-response discussed above. In any given cell that expresses particular receptors, there are literally billions of receptor molecules, existing in either an 'on' (agonist is bound to the receptor) or

'off' (no agonist is bound to the receptor, or it is occupied by an antagonist ligand) position. The magnitude of the biological response that occurs following ligand activation of a particular receptor type (e.g., estrogen receptor, AhR, CAR, etc.) is a function of the percent of total receptors occupied at the same time. In other words, there is a dose-response relationship between ligand concentration and the number of receptors that

Figure 5. Dose-response relationship for activation of the estrogen receptor by different activating ligands (from: CosmoBio, 2015)



are occupied. Typically, dose-response relationships for receptor-mediated responses are very steep, meaning that, at the low dose end of the curve, there is no measurable biological response in an individual until the dose becomes high enough to occupy a significant percentage of all of the receptors in a particular cell. Once the concentration (which is a function of dose) of chemical in the tissue reaches a level adequate to occupy a significant percentage of receptors, further increases in dose/concentration cause dramatic increases in the magnitude of response. In other words, the dose-response curve is very steep, and shows clear 'thresholds' at the lower end of the dose range, for estrogen activation of the estrogen receptor by estrogen and other estrogen receptor agonists (Figure 5). A threshold simply means that the concentration of agonist in the target tissue is too low to cause a biologically meaningful response. The importance of the shape of the dose-response curve for PCBs will be discussed in detail in a later section.

Another important concept in understanding the toxicology of molecules like PCBs is that of 'potency.' While multiple different chemicals might have the ability to interact with the same receptor, they may differ drastically in how much of the chemical it takes to cause a biologically relevant activation of the receptor. This is one of the most important concepts in the pharmacology of drugs that are designed to interact with specific receptors, since the effective dose is dependent upon both the amount of chemical administered (the dose) AND how potent it is in interacting with the receptor. For example, there are multiple different steroid drugs that are widely used to treat inflammation and a variety of other human conditions by altering a receptor known as the 'glucocorticoid receptor.' These are frequently referred to as 'steroids' in the lay literature, but there are actually very specific forms of steroid molecules that interact specifically with one type of the glucocorticoid receptor. Drugs like prednisone, dexamethasone, and hydrocortisone all interact with and activate glucocorticoid receptor(s), but the 'equally effective doses' for each of these are:

Hydrocortisone, 20 mg; Dexamethasone, 0.75 mg; Prednisone, 5 mg. Thus, even though they act the same way (activate the corticosteroid receptor), it takes about 27 times more hydrocortisone than dexamethasone to cause the same effect (dexamethasone is 27 times more potent than hydrocortisone). So, too, different forms of the same class of environmental chemical can have vastly different potency in causing an effect through a receptor.

The difference in potency is well documented for the DL-PCBs, where one specific PCB, PCB126, is highly potent in activating the rat AhR, yet PCB118 is a very weak activator, taking almost 10,000 times more PCB118 molecules to activate the same number of rat AhR receptors as one molecule of PCB126. Obviously, adjusting for the different potency of different forms of PCBs toward the AhR is necessary when looking at environmental mixtures. Following the lead of toxicologists who recognized this important difference in PCB molecules in the 1980s, the World Health Organization in 1998 established 'Toxic Equivalency Factors' (TEFs) to allow for adjustment for potency in mixtures of DL-PCBs.

There are also large species differences between how the AhR interacts with dioxin-like molecules, so if the concern is over potential adverse effects of humans, it is essential to adjust for species differences in the potency of different dioxin-like molecules to interact with the human AhR. As noted above, there are also well known species differences in CAR and PXR receptors (Tabb *et al.*, 2004) which must be taken into account when using responses seen in laboratory animals to predict effects in humans. These points are discussed below and in detail in Appendix 3.

4. Formation of 'reactive intermediates' as a mode of action in toxicology

Another way that chemicals, both endogenous and exogenous, can cause toxic effects in the body occurs through a process called biotransformation. Evolution has generated a remarkable process by which living organisms can adapt to potentially toxic substances in their environment. The DNA in every human (called the human genome) codes for the production of several thousand different proteins, collectively referred to as 'biotransformation enzymes' (often called 'drug metabolizing enzymes', although they metabolize many chemicals in addition to drugs). Although one of the primary purposes of biotransformation is to rid the body of chemicals foreign to the body, in so doing the body can actually make the chemical *more* toxic, not less. Such products of biotransformation are referred to as 'reactive intermediates' because they are usually very short-lived in the body, precisely because they are reactive, and tend to bind to proteins and sometimes DNA, in the cell where they were generated. Sometimes the 'reactive intermediate' molecules actually generate more reactive molecules in the body, causing a multiplying cascade of more reactive intermediates. Usually these reactive intermediates involve the generation of forms of oxygen that themselves are highly reactive, a process referred to as 'oxidative stress'. The body is well equipped with protective pathways to deal with these reactive forms of oxygen, since even normal metabolism of sugars (glucose) in the body generates them. However, at high doses of a foreign substance that generates oxidative stress, these protective pathways can be overwhelmed, and the ensuing oxidative stress can result in serious toxic responses, including organ failure and death. Perhaps the best example of this is with the widely used over-the-counter-medication, acetaminophen (Tylenol). At normal therapeutic doses of acetaminophen (e.g., less than 10 grams per day), a small fraction of the dose is metabolized to a reactive intermediate, but it is quickly sequestered and made non-toxic by the liver. However, if the maximum recommended daily dose is exceeded, these protective mechanisms are overwhelmed, and serious toxicity to the liver can occur.

Indeed, acetaminophen overdose is a leading cause of liver failure in the United States, yet millions of people take this compound safely every day because the dose is below the ‘threshold’ for liver damage. As with receptor-mediated responses, generation of reactive intermediates from foreign molecules generally follows a steep dose-response curve, with clear evidence of thresholds at the lower doses. There is extensive evidence in experimental animals that high doses of PCBs can induce oxidative stress (see Appendix 3: Cancer and mode of action for PCBs), and this likely contributes to most if not all of the liver toxicity seen in studies in both animals and humans exposed occupationally to high doses of PCBs.

5. The special circumstance of ‘reactive intermediates’ that bind to and damage DNA:

Some biologically reactive intermediates formed in the process of metabolizing the chemical to non-toxic forms can bind to and damage DNA. These so-called ‘genotoxic’ chemicals are often treated differently in the arena of regulatory toxicology, because early studies conducted with ionizing radiation (itself a form of exposure to a highly reactive form of energy), led to a theory that the risk of damage to DNA was directly proportional to the amount of exposure even at very low doses. These studies led to the development of the so-called linear, non-threshold (LNT) dose-response relationship for cancer. This theory, now largely debunked, served as the basis for regulation of a large number of chemicals found to cause cancer in laboratory animals through a ‘genotoxic’ mode of action. The US EPA’s evaluation of the carcinogenic actions of PCBs completed in 1996 presumed that the mode of action for causing liver tumors in rats might be related to genotoxic effects of PCBs, and thus used the ‘linear, non-threshold’ approach to regulate them. Although there is now overwhelming scientific evidence that demonstrates that this assumption for PCBs is incorrect (reviewed in detail later), it continues to serve as the basis for most regulatory actions related to PCBs in the environment, including the establishment of clean-up goals for the Spokane River.

6. Using chemical-specific dose-response data to determine ‘safe’ levels of chemicals in the environment

The field of *chemical risk assessment* is a derivative of the science of toxicology and relies upon the scientific understanding of exposure (how, and how much), hazard (the type of adverse effect(s) that might occur, and potency (how much it takes to cause the hazard to become real). The standard approach used for decades follows a paradigm that was validated for regulatory purposes through a consensus report from the National Academy of Sciences in 1983 (National Research Council, 1983). The basic approach for assessing the risk of chemical hazards has not fundamentally changed since this report, although numerous improvements in incorporating new science into each step along the way have been made. The general paradigm is captured simply in the following equation:

$$\text{Risk} = \text{exposure} \times \text{hazard}$$

Where ‘Risk’ is a probability estimate of the likelihood that an adverse outcome will occur in a population of people exposed to a substance with known/suspected hazard. ‘Exposure’ is a quantitative measure of dose, usually expressed in units of milligrams [mg] of substance per kilogram (kg) of body weight per day, and for chronic effects such as cancer generally assumes a lifetime of exposure. ‘Hazard’ reflects the nature of the adverse outcome (e.g., cancer, birth defects, mortality, liver disease) AND some measure of potency – how much of the chemical (dose) is required to cause the hazard to become real. But this equation over-simplifies the process, which is illustrated more completely in Figure 6 from the 1983 NAS ‘Red Book’:

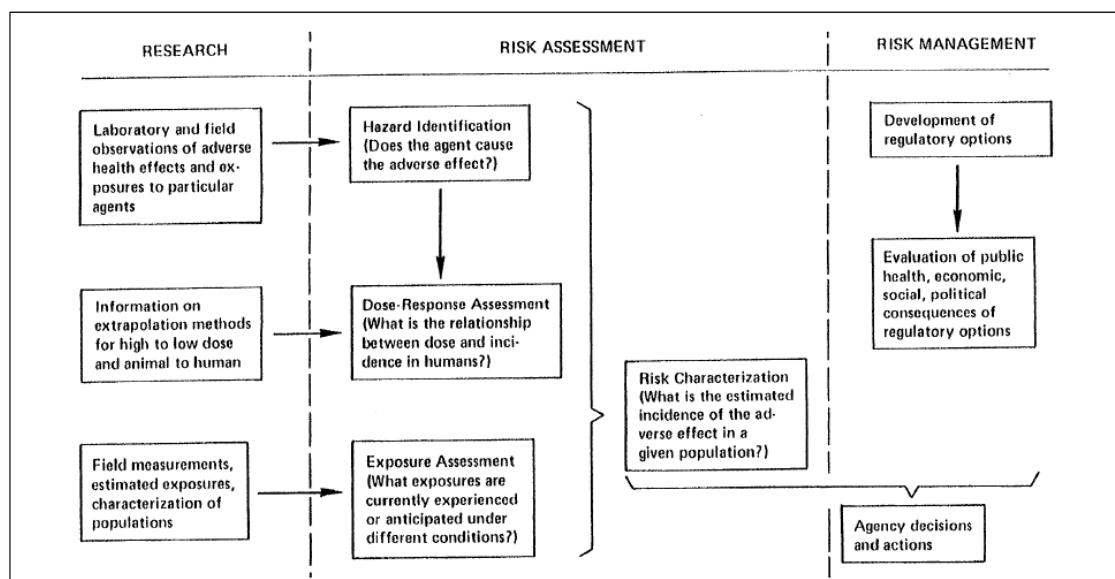


Figure 6. Elements of Risk Assessment and Risk Management (from: National Research Council, 1983)

Looking within the risk assessment process (center box), it is evident that there are three components to risk – hazard e.g., does the chemical cause the effect in question?, Dose-response assessment, which is determining the dose, usually in mg/kg-day (d), that is capable of causing the adverse outcome in a population (of humans, or of experimental animals) and the all-important third step, which is to estimate actual human exposures, in the same dose units (md/kg-d), under a defined set of conditions. Once all three of these steps have been completed, it is then necessary to pull it all together in the form a ‘risk characterization’, which basically uses the dose-response data to project an incidence (which, for cancer risk for genotoxic carcinogens, is often expressed as number of theoretical cases of cancer per million population, e.g., 5×10^{-6} would mean that an exposure to substance x at a daily dose of y md/kg-d, every day of their lives for 70 years, would result in no more than 5 additional cancer cases per million people exposed according to the exposure scenario). To put that risk in perspective, the American Cancer Society estimates that the lifetime risk of getting some form of invasive cancer is 39.66% for men, and 37.65% for women, and the lifetime chances of dying from some form of cancer is 22.03% for men, and 18.76% for women (American Cancer Society, 2018). Thus, for example, a 5 in a million (5×10^{-6}) increased lifetime risk of getting cancer would mean the risk for men would go from 39.66% to 39.6605%, and for women, it would go from 37.65% to 37.6505%.

For nearly all other adverse outcomes (e.g., for all effects except for cancer risk following exposure to DNA-damaging chemicals), the goal is to identify a point on the ‘dose-response curve’ below which no adverse response would be expected. This is the so-called ‘threshold’ dose- e.g., a daily dose below which no adverse response would be expected, even in a large population exposed daily to that dose for a lifetime. Once reliable estimates of both human ‘dose’ and the animal ‘threshold’ dose (usually called ‘Benchmark Dose’) are established, Margins of Exposure can be calculated to determine if a particular human exposure scenario

is likely to result in a dose that is toxic. Of course, many assumptions go into this process that need to be discussed, and where possible, validated. That is part of the process of risk characterization.

In the risk assessment paradigm discussed in the 1983 NAS Red Book (National Research Council, 1983), it is the shape of the population dose-response relationship that is analyzed, with an attempt to identify the lowest dose that is without affect, or 'point of departure', in a population (animal or human)– the threshold dose. This is typically done for animal toxicology studies where groups of rats or mice are given different doses, usually over a period of 90 days, although studies for cancer are usually done for the lifetime of the animals (2 years). If the substance in question causes more than one type of adverse effect, scientists establish a 'point of departure' or benchmark dose for each type of adverse outcome that was observed. Once dose-response analysis has been completed and apparent threshold doses (Point of Departure, Benchmark Dose, NOAEL) have been established, the next step is to conduct an exposure analysis to determine what the human dose might be, under a defined set of conditions. So, for chemicals such as PCBs found in trace levels in the diet, the exposure analysis would require knowledge of 1) what the concentration of the chemical of interest is in the food item(s) consumed (usually expressed as micrograms (ug) of chemical per kg of food item, which is equivalent to 'parts per billion', or ppb), and 2) a reasonable estimate of the quantity of the food item(s) consumed on an average daily basis (usually expressed as g/day). This then allows one to calculate a daily dose, expressed in 'mg of chemical per kg of body weight per day, mg/kg-d, the same units that are used to express the estimate of the threshold dose (Benchmark Dose, NOAEL, Point of Departure) identified in animals. This step usually includes some adjustments to correct for species differences in dose units. For example, it is generally recognized that dose-response relationships 'scale' better when going from small animals like rats and mice, to large animals like humans, if dose is expressed in units of mg of chemical per square meter of body surface area (mg/m² BSA), than mg/kg body weight. There are relatively simple ways to make this adjustment for cross-species comparisons, called 'scaling factors.'

Once both an estimate of human exposure and a threshold dose ('Point of Departure' or 'Benchmark Dose') from the animal studies (for each end point) are obtained, the standard approach is to simply calculate the ratio of the human exposed dose to the rat threshold dose. This ratio is called a Margin of Exposure (MOE), and simply represents how close, or how far away, the human dose is to the highest dose in animals that didn't cause evident toxicity. If the ratio is 1, that means that, under the conditions of exposure defined in the human exposure assessment, humans would be receiving a dose that was very close to the threshold dose, so there would be some likelihood that some fraction of humans in an exposed population might well have an adverse response. The larger the MOE, the less unlikely it becomes that someone in a human population exposed according to the exposure scenario used to estimate dose would have an adverse response. Because there is usually some uncertainty about whether humans and rodents have similar susceptibility to the toxic substance in question, risk assessors have traditionally used 'safety factors', or 'uncertainty factors', to allow for the possibility that humans might be more susceptible than laboratory animals (even after adjusting for body weight to body surface area differences – i.e., after use of scaling factors). Typically, a factor of 10 is used for cross-species extrapolation from rodents to humans. However, if there are experimental data that can demonstrate that humans are more, or less, susceptible than the experimental animals, those data should be used, rather than the 'default' factor of 10 species adjustment. Because not all humans will have the same susceptibility, another 'uncertainty factor' of 10 is often used to adjust for 'interindividual differences' in the human population, since the goal is to protect even the most

sensitive individuals in a population. Additional ‘uncertainty factors’ may be added on, depending on the amount and quality of the data available, and the level of understanding of ‘mode of action’ for the toxic response. Thus, MOEs that are less than 100 are generally not considered to be protective of public health, whereas MOEs greater than 100 (or possibly more, depending of the nature of the data and the ‘risk characterization’) are generally viewed as having ‘*deminimus*’, or acceptable, risk’, which is essentially equivalent to the lay term, ‘safe’ (Eaton and Gilbert, 2013; Faustman and Omenn, 2013).

In recent years great advances have been made in developing more scientifically robust ‘Points of Departure’ (or Benchmark Doses) based on mode of action/mechanistic data, using a process called ‘Adverse Outcomes Pathway Analysis’ (AOP). This is especially useful for chemicals for which the mechanism(s) of action (exactly how the chemical causes its adverse effect) are well understood. Because of the enormously rich body of knowledge on the molecular toxicology of PCBs, they are ideal candidates for an AOP approach. A review of the Adverse Outcomes Pathway approach to chemical risk assessment is provided in Appendix 3.

This approach, endorsed and encouraged by scientists from the EPA (Kleinstreuer *et al.*, 2016), has been used in this expert report. Appendix 3 provides a detailed assessment of the AOP approach as it pertains specifically to estimating Points of Departure (POD; Benchmark Dose) for toxicological outcomes from PCBs that are driven by one specific mode of action, activation of the ‘Aryl hydrocarbon Receptor’ (AhR). More traditional MOE analyses have also been conducted for several toxicological end-points for which the available evidence suggests that the mode-of-action is not solely via activation of the AhR.

Using an AOP approach I have determined the ‘threshold dose’ or Point of Departure in animals studies for outcomes for which there is compelling data that extensive and prolonged activation of the AhR by the ‘dioxin-like PCBs’ is essential (the various types of PCB molecules that have similar, or different, ‘modes of action’ are discussed briefly in the next section). These outcomes include cancer, immune toxicity, and most adverse reproductive and developmental outcomes. Margins of Exposure have then been calculated for theoretical cancer risk (Appendix 3: Cancer and mode of action for PCBs), using the dose in rats that is associated with 20% activation of the AhR in rat liver animals (the Benchmark Dose), since there is overwhelming scientific evidence that this level of activation does not increase cancer incidence in laboratory. For immune response and most reproductive and developmental outcomes for which overwhelming evidence demonstrates that activation of the AhR is essential for the toxic response, I have used Benchmark doses in animal studies that have been adjusted for the well-known species difference in AhR activation by DL-PCBs (Appendix 4: Immunotoxicology of PCBs; Appendix 6: Reproductive toxicology of PCBs). I have then conducted an exposure analysis using PCB concentrations measured in edible fish tissues from the Spokane River, coupled with fish consumption survey data of people who routinely harvest fish for consumption from the Spokane River (Section II.A, Appendix 2: Exposure assessment). I then use these data to conduct a Margin of Exposure Analysis to determine if PCBs present in fish in the Spokane River represent an unreasonable risk to consumers of those fish (or, to determine if consuming typical amounts of fish harvested from the the Spokane River is ‘safe’).

7. What are PCBs?

These topics have been discussed in great detail in other expert reports, so I will only summarize some key points that are relevant to my evaluation of whether fish harvested from the Spokane River containing trace

levels of PCBs are safe for human consumption (which utilizes the standard approaches for risk assessment outlined above).

PCBs found in the environment are a mixture of specific types of molecules, with 209 possible forms, called 'congeners.' As discussed briefly above (and in detail in Appendix 3), a small sub-set of these congeners have the ability to interact with a protein molecule called the 'Aryl hydrocarbon Receptor', or AhR for short. There are 12 PCB congeners that are able to bind to and activate the rat AhR. These are called 'dioxin-like PCBs' (DL-PCBs) since dioxins (a group of molecules that share some common structural features with these PCBs) are potent activators of the AhR. This is very important, since it has now been demonstrated that many, but not all, of the toxic effects of PCBs seen in laboratory animals are due to these DL-PCBs. As will be discussed in great detail later, there is now an extensive body of scientific literature demonstrating that the ability of some PCB mixtures to cause the liver cancers seen in some 2-year rat cancer studies is due solely to this sub-set of DL-PCBs, although some of the other PCBs at high doses may modify the potency of the DL-PCBs in causing liver cancer in rats. In recent years several important studies have compared human liver with rat liver, and have demonstrated that the human AhR is much less responsive to activation by DL-PCBs, when compared to rat liver. **Indeed, only one of the 12 DL-PCBs that activate the rat AhR was found to have any measurable ability to activate human AhR.** (This important point is discussed in detail in Appendix 3). But it is recognized that some of the toxic effects of PCB mixtures observed in laboratory animals are not mediated via the AhR, so these must be considered differently.

PCBs are fat soluble and resistant to environmental degradation, and we now recognize that these two properties are largely responsible for the presence of PCBs in the environment, and in the food chain, including trace levels found in fish in the Spokane River. The focus of much of the remainder of this report is to evaluate whether the presence of these PCBs represent any significant health risk to consumers of fish harvested from the Spokane River (i.e., are fish in the Spokane River safe to eat?). It stands to reason that, if current levels of PCBs in fish represent no significant risk to the small fraction of the population that consumes these fish, that efforts to lower the concentration of PCBs in the Spokane River, even if successful, will have no public health benefit, even to those who consume relatively large amounts of fish harvested from the River. If, on the other hand, a 'Margins of Exposure' (MOE) analysis using data available today indicates that MOE for some endpoints are not adequate (e.g., they are determined to be less than 10 - 100 for the top consumers of fish from the Spokane River), then revised fish advisories and increased public communication targeted to high end consumers might be of some public health benefit to the small fraction of the population that utilizes fish from the Spokane River as a significant portion of their diet.

8. Summary of approach to assessing the relative risk (or virtual safety) of PCBs in fish from the Spokane River

As discussed above, in order to determine whether something is "safe for consumption", one must first have an estimate of 'dose', or how much a person is exposed to, and how frequently. This dose is then compared with the doses used in experimental animal studies where specific types of adverse effects (response) were observed. From the animal studies, it is usually necessary to have data where a range of different doses was used, including doses that produce the effect, and a lower dose that does not produce the effect. Establishing a good estimate of the dose in experimental animals that has little or no toxic effect (the Point of Departure, POD, discussed above) is used for comparison of estimates of actual human exposure under a

defined set of conditions (e.g., concentration of specific PCB congeners in fish, amount of fish consumed on a daily basis) to calculate Margins of Exposure (MOEs). MOEs of greater than 100 - 1000 are often needed to assure human safety.

Doses in humans that are close to the POD dose in animals (i.e., MOEs of less than 10), would generally not be considered to be safe if exposures are likely to occur for many years, and/or there are a significant number of potentially 'sensitive' sub-groups (e.g., pregnant women, infants and children) in the exposed population.

As my expertise is in toxicology, I have limited my analysis to toxicological literature, and have relied upon other qualified experts in epidemiology to assess the human epidemiological literature, also using a 'weight of evidence' approach. Thus while I did not myself critically evaluate each of the many epidemiological studies that have been performed for various outcomes (possible toxicological effects) between environmental and/or occupational exposures to PCBs, I did consider the opinions of experts in epidemiology who have thoroughly and critically evaluated that body of literature, and made weight of evidence assessments of the likelihood of **causal** connections between exposure to PCBs and particular disease outcomes, based on available human epidemiological evidence.

9. Steps required to make an assessment of risk/safety for a specific exposure scenario

For the assessment of each possible adverse outcome from PCBs found in fish in the Spokane River, based on toxicological studies in experimental animals, the following steps are required:

1. **Evidence from experimental animal studies** that PCBs are able to reproducibly cause the outcome of interest (Hazard assessment).
2. **Current state of knowledge** about the Mode of Action (MoA) of PCBs for causing the outcome of interest in laboratory animal studies, and evaluation of data regarding the relevance of the MoA to humans, if available.
3. **Dose-response analysis** for the outcome of interest in experimental animals, to determine the most reliable 'Point of Departure' (POD)/Benchmark dose for that specific outcome of interest.
4. **Site-specific exposure assessment** to provide estimates of both expected (mean) and upper bound (95th percentile) daily exposures to PCBs of interest through fish consumption (this will be the same for all outcomes of interest).
5. **Risk characterization**, including dose adjustments for cross-species extrapolation, including standard 'scaling factors' for body weight corrections, and mechanism-based knowledge of species differences in response for each outcome, if available.
6. **Calculation of Margins of Exposure (MOE)** for each outcome, which is the ratio of the estimated daily exposure, in mg/kg/day (with appropriate dose adjustments for species differences) to the Benchmark Dose determined for each type of adverse outcome. Benchmark Dose refers to the highest daily dose that had no, or minimal, effects in an animal study. For 'weight of evidence' analyses, it is important to use ALL available data that meets minimum study design criteria. The EPA has provided general guidance on various approaches to calculating 'points of departure' for use as Benchmark Doses (US EPA, 2012a). However, where robust 'mode of action' data are available, a

newer approach, called 'Adverse Outcomes Pathways' (AOP) approach, is more scientifically robust, and has been used in this report for assessing possible adverse health outcomes from PCBs for endpoints where activation of the Arylhydrocarbon Receptor (AhR) has been demonstrated to be the mode of action. Appendix 3 provides a detailed description of the AOP approach, and how it can be used to determine MOEs for toxicological effects that are mediated by DL-PCBs.

Once an MOE has been calculated for a specific outcome, it is common practice to apply uncertainty factors (UF), sometimes called safety factors, to the actual calculated MOE before making judgements about safety. Where no biologically relevant information is available on possible species differences between the experimental animals and humans is available, a UF of 10 is often applied (assumes humans may be 10 times more sensitive than the test animals used in the toxicology study). However, where mechanistic data are available to adjust for species differences, those should be used in place of the default factor of 10 for cross-species comparisons². Another factor of 10 is frequently used to accommodate potential inter-individual differences among the human population, and additional UFs might be applied if data are limited, or, as noted above, if the Point of Departure is based on a 'Lowest Observable Effect Level' (LOAEL) rather than a NOAEL or modelled Benchmark Dose. As general guidance, a MOE of 100 or less would be considered to represent an unreasonable risk of the adverse outcome occurring in a population exposed according to the exposure scenario described; an MOE from 100 -1000 would be considered to represent an acceptable level of risk for all but the most highly exposed populations, and an MOE of greater than 1,000 would represent a '*deminimus*' risk level, equivalent to a virtually safe dose- e.g., even high consumers of fish would have no measurable adverse effect from the exposure, even if it occurred over a lifetime.

² US EPA (2005). Guidelines for Carcinogen Risk Assessment. EPA/630/P-03/001F. March. "Relative potency factors (of which toxicity equivalence factors are a special case) can be used for a well-defined class of agents that operate through a common mode of action for the same toxic endpoint. A complete dose-response assessment is conducted for one well-studied member of the class that serves as the index chemical for the class. The other members of the class are tied to the index chemical by relative potency factors that are based on characteristics such as relative toxicological outcomes, relative metabolic rates, relative absorption rates, quantitative SARs, or receptor binding characteristics (U.S. EPA, 2000c). Examples of this approach are the toxicity equivalence factors for dioxin-like compounds and the relative potency factors for some carcinogenic polycyclic aromatic hydrocarbons. Whenever practicable, toxicity equivalence factors should be validated and accompanied by quantitative uncertainty analysis."

II. Scientific evaluation of the toxicological risks of PCBs found in edible sport fish in the Spokane River

A. Exposure assessment for PCBs in edible fish in the Spokane River (see Appendix 2 for supplementary materials)

Although this is the third step in the process outlined above for establishing the safety of exposures to a potentially toxic substance in the environment, it is discussed here first, because the exposure analysis will be the same for all toxicological end-points considered in later sections of this report.

1. What are the concentrations of total PCBs and specific PCB congeners of toxicological interest in fish in the Spokane River?

As described below, data were retrieved from publicly available sources and compiled to derive estimates for the concentrations of individual congeners as well as the concentrations of total PCBs in edible fish in the Spokane River.

Data for concentrations of total PCBs and specific PCB congeners of toxicological interest in Spokane River fish tissue were compiled from available raw data collected by the Washington State Department of Ecology (ECY). These data are publicly accessible through the Environmental Information Management (EIM) database (WA Department of Ecology, 2019).

EIM data were extracted from the database using the advanced search feature. Parameters engaged to selectively retrieve data from EIM included (1) Location – County³: Spokane, Stevens, Lincoln; (2) Result – Result Parameter (contains) “PCB”; and (3) Result – Sample Matrix: Tissue. These parameters resulted in 33,270 entries from 97 unique sample locations. Results were further restricted to skin-on fillet samples (not gut contents or whole fish) from fish collected from the Spokane River whose analysis quantitated PCB congeners rather than total PCBs or Aroclors. These restrictions allowed for examination of congener-specific PCB concentrations in the fish tissue most relevant to Spokane River fish consumers.

The resultant compiled dataset encompassed tissue samples collected for 4 different species of fish collected between 1999 and 2012. The compiled dataset included data attributed to the Washington State Toxics Monitoring Program (2003, 2012), Spokane River PCB Source Assessment (2003), Metals and PCBs in Long Lake Fish (2001), and Spokane River fish and crayfish PCBs and Metals (1999).

All but one of the studies retrieved above used EPA1668 as the congener analysis method and analyzed all 209 congeners. The single study that employed a different analytical method was the 1999 analysis that reported quantitation of 24 PCB congeners using method SW8082, which is designed for quantitation of PCB mixtures and 19 PCB congeners (US EPA, 2007). For consistency and accuracy, the 1999 assessment was excluded from the final analysis; results were from samples collected twenty years ago quantitated (with a

³ Due to varying location naming conventions used in the EIM database, the location parameter was restricted by County rather than by body of water or watershed to ensure all available Spokane River fish tissue data was included. The search was limited to Spokane, Stevens, and Lincoln counties, which encompass the entirety of the Spokane River from the Idaho border to the Columbia River.

non-standard group of congeners) using a different method than all more recent analyses that were included in the assessment.

For the evaluated dataset, individual PCB congeners with concentrations reported as below the limit of detection were assumed to be zero, which is consistent with the standard method of handling non-detect values for PCBs (US EPA, 2000; WA Dept of Ecology, 2018). PCB congeners that were detected, but not quantified (i.e., reportedly between the detection limit and reporting limit) or tentatively identified were included in this analysis as the estimated value provided in the database.

There was one instance whereby the EIM database reported results from a replicate (or duplicate) analysis. In the 2003 Washington State Toxics Monitoring Program study, EIM sample IDs 3084282 and 3084308 were identified as resulting from a split of a rainbow trout tissue sample prior to laboratory analysis. To avoid double counting this sample, this assessment combined the replicate results into a single sample with congener concentrations assigned as the mean of the concentrations reported in the replicate analyses.⁴

The EIM database included tissue concentration results for samples labeled as “rainbow trout, redband trout, steelhead” because they share the common scientific name of *Oncorhynchus mykiss*. However, because steelhead are anadromous trout (i.e., they migrate and live in oceanic waters before returning to fresh water to breed), steelhead tissue concentrations would not be reflective of Spokane River conditions and therefore, like anadromous salmon, steelhead concentrations are not appropriate to include in an assessment of Spokane River fish. Although the EIM data submission instructions indicated any samples entered with an *Oncorhynchus mykiss* designation required a field collection comment to document whether the life history was anadromous (steelhead) or resident (rainbow trout), none of the *Oncorhynchus mykiss* samples included such a designation. To ensure steelhead samples were not included in the analysis, each dataset retrieved from the EIM that reported the sample type as “rainbow trout, redband trout, steelhead” was reviewed. As summarized below in Table 1, all sample types were confirmed to have been Rainbow trout.

⁴ Congener concentrations reported below the limit of detection were assumed to be zero. If only one of the two duplicate samples were below the limit of detection, the value of the detected sample was used rather than an average of the two.

Table 1. Rainbow trout sample confirmation

Confirmed sample type	EIM Study ID	EIM Year	EIM Sample IDs	Rationale
Rainbow trout	WSTMP03T	2003	3084281 - 3084296, 3084288 - 3084289, 3084301 - 3084306, 3084308	Sample IDs (without the preceding 30-, which was presumed to indicate sampling year) correspond with "Rainbow trout" tissues in Table 32 of Spokane River PCB Source Assessment 2003-2007 (WA Dept of Ecology, 2011)
Rainbow trout	DSER0010	2003	4188308, 4188309	Sample IDs (without the preceding 41-, which was presumed to indicate sampling year) correspond with "Rainbow trout" tissues in Table 32 of Spokane River PCB Source Assessment 2003-2007 (WA Dept of Ecology, 2011)
Rainbow trout	WSTMP12	2012	1301011-80 - 1301011-88	Department of Ecology indicated Spokane River trout samples collected and analyzed in 2012 were "Rainbow trout" (WA Dept of Ecology, 2014)

In total, there were 59 unique samples from largemouth bass, largescale sucker, mountain whitefish, and rainbow trout that met the criteria of congener-specific fillet samples from the Spokane River. Figure 7 shows the location for congener-specific fish tissue samples meeting these criteria.

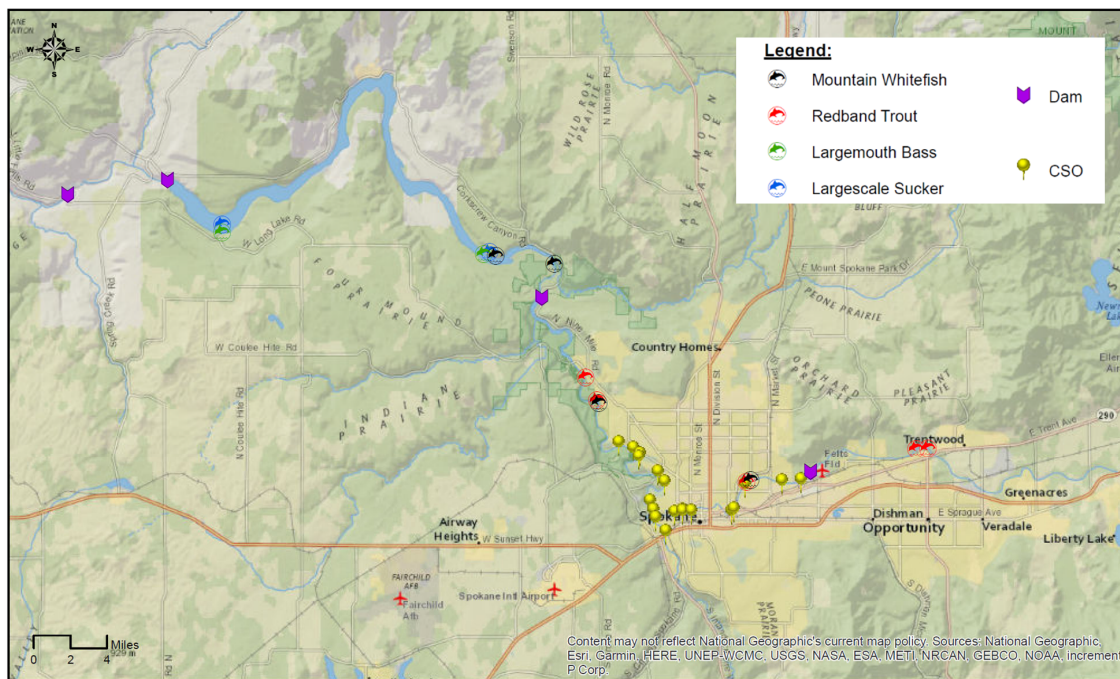


Figure 7. Location for congener-specific fish tissue samples. Includes locations of dams and combined sewer overflow (CSO) stations

All fish concentration data was reported and incorporated in terms of mass per grams of uncooked fillet weight. No correction was applied to loss of PCBs that occurs upon cooking even though a recent review indicated the median loss across 14 studies ranged from 25 to 39% (AECOM, 2012).

Estimation of species-specific concentrations for detected PCB congeners

Congener-specific PCB concentrations across all studies and survey years were used to calculate the mean concentration for each congener in each species of fish. For a given species and study, the mean concentration of a given PCB congener (PCB_x) was calculated as:

$$\bar{X}_{PCB_x} = \frac{\sum_{c=1}^n [PCB_x]_c}{n_c}$$

where \bar{X}_{PCB_x} is the species-specific mean concentration of a given PCB congener, c is the concentration of a given PCB congener in a sample for a given species, and n_c is the total number of samples that analyzed for that congener for a given species. Total PCB concentrations were calculated for each species by taking the mean of the sums of all measured congeners for each sample. This method was used to account for differences in congener analysis (e.g., congener co-elution) between studies.

This exposure assessment focuses on fish tissue concentration data within and downstream of Spokane city limits, as those are the locations that would potentially be affected by the proposed remediation efforts. Thus, the two trout samples upstream of the upriver dam in Figure 7 (e.g., between the upriver dam and the Idaho border) were not included in the mean PCB concentrations for rainbow trout.

Table 2. Tissue concentrations of total PCBs for fish in the Spokane River by species.

Common name	Species	Study ID	Sampling Year	Number of Samples	Mean Total PCBs (ng/g)
Largescale sucker	<i>Catostomus macrocheilus</i>	RJAC002	2001	6	127
Largemouth bass	<i>Micropterus salmoides</i>	RJAC002	2001	6	68.3
Rainbow trout	<i>Oncorhynchus mykiss</i>	WSTMP03T	2003	24	36.4
		WSTMP12	2012	6	
Mountain whitefish	<i>Prosopium williamsoni</i>	RJAC002	2001	3	118
		WSTMP12	2012	9	

2. What are the best estimates of fish consumption rates for fish harvested from the Spokane River?

Estimation of fish consumption rates

Dr. Sunding estimated fish consumption rates (FCRs) by species for three adjacent Washington counties adjacent to the Spokane River. As specified in Dr. Sunding's Expert Report, estimates were calculated based on population demographics and responses to the Industrial Economics (2013) Upper Columbia River Survey. Table 3 details Dr. Sunding's estimated FCRs among fish consumers. Because these FCRs reflect consumption

from a wider geographical area than just the Spokane River, they represent conservative (likely to overestimate) estimates for Spokane River FCRs.

Table 3. Fish consumption rates by species among fish consumers (table from Dr. Sunding's Expert Report)⁵

Sub-Population			Fish Consumption Rates among Anglers (g/day)				
			Mean	Quantiles			
				50%	90%	95%	99%
Walleye	[1]	51.4%	2.25	0.47	4.66	9.94	22.37
		[45.9%, 57.3%]	[1.57, 3.12]	[0.00, 0.93]	[3.11, 9.32]	[7.46, 15.53]	[15.53, 27.96]
Rainbow Trout	[2]	33.0%	1.45	0.00	4.66	7.77	15.53
		[27.8%, 37.6%]	[0.95, 2.04]	[0.00, 0.93]	[3.11, 7.46]	[5.56, 9.32]	[12.43, 23.30]
Bass	[3]	7.2%	0.31	0.00	0.62	1.24	4.66
		[4.5%, 10.6%]	[0.15, 0.58]	[0.00, 0.00]	[0.40, 0.95]	[0.62, 2.33]	[2.49, 13.98]
Kokanee	[4]	6.2%	0.27	0.00	0.93	1.86	6.21
		[4.2%, 8.0%]	[0.14, 0.43]	[0.00, 0.00]	[0.00, 1.43]	[1.18, 3.11]	[3.73, 9.32]
Other	[5]	2.2%	0.10	0.00	0.00	0.00	1.35
		[1.0%, 4.0%]	[0.04, 0.22]	[0.00, 0.00]	[0.00, 0.00]	[0.00, 0.62]	[0.78, 1.86]
All	[6]	100%	4.38	1.86	10.10	16.78	38.84
		[100%, 100%]	[3.42, 5.44]	[1.10, 2.49]	[8.08, 15.53]	[12.43, 21.75]	[27.77, 46.60]

As specified in Table 3, Spokane River fish consumption consists of primarily four species of fish (e.g., kokanee, rainbow trout, walleye, and bass). However, as specified above, while Spokane River fish congener-specific tissue analyses have included rainbow trout and largemouth bass, they have not included kokanee or walleye. Kokanee and walleye tissue concentrations were estimated using a surrogate approach. Specifically:

1. Kokanee PCB congener concentrations were estimated using rainbow trout as a surrogate. Rainbow trout and kokanee are both from the genus *Oncorhynchus* and have relatively similar diets and habitats. Thus, the tissue concentration of PCB congeners in rainbow trout were applied to both kokanee and rainbow trout.
2. Walleye PCB congener concentrations were estimated using largemouth bass as a surrogate. Largemouth bass and walleye are both predator fish with similar diets and habitats. Historically, walleye have exhibited similar mean tissue PCB concentrations (U.S. Fish and Wildlife Service, 2019a, b; US EPA, 2000). Thus, the tissue concentration of PCBs in largemouth bass were applied to both walleye and bass.

The "other" category in Table 3 includes additional species of fish consumed from the Spokane River. Fish tissue PCB concentrations for species in the other category were conservatively estimated using congener tissue concentrations of Mountain Whitefish.

⁵ From Dr. Sunding's expert report: This table calculates the average species composition of fish consumed from the Spokane River in three adjacent Washington counties. Estimates are calculated based on the population demographics in Table 3 and responses to part D of the Industrial Economics (2013) Upper Columbia River Survey. Fish are categorized into groups as presented in the original survey. This table also calculates the quantiles of the fish consumption rate for specific species on the Spokane River. The model assumes that rates of species-specific fish consumption are similar among consumers on the Spokane River as on Lake Roosevelt within each demographic control group. Standard Errors in square brackets are calculated by bootstrap. Fish consumption quantile estimates are projected onto the population demographics reported in Table 3.

What are the best estimates of PCB intake rates for fish harvested from the Spokane River?

Species-specific fish consumption rates presented in Table 3 were combined with compiled concentrations of congener-specific PCBs to estimate human PCB intake for both individual congeners of interest and for total PCBs:

$$[PCB]_{intake} \left(\frac{ng}{day} \right) = \sum_{s=1}^n PCB_s \cdot FCR_s$$

Where $[PCB]_{intake}$ represents the concentration of an individual congener (or total PCBs) consumed daily. As described in the equation above, daily intake was derived from the multiplicative product of PCB_s (species-specific concentration of either a single congener or total PCBs in ng/g fish) and FCR_s (species-specific daily human consumption rate in g fish/day); multiplicative products were then summed for all species considered. Intake rate was estimated for several different scenarios, determined by the use of either mean or 95th percentile species-specific tissue concentrations and fish consumption rates (see Table 4 below).

As described in subsequent sections evaluating potential adverse effects (e.g., cancer, immunotoxicity, neurodevelopmental, reproductive or developmental effects), margins of exposure (MOEs) compared estimated PCB intake for a specific congener (or congeners) to exposure concentrations evaluated in studies described in toxicological literature. Congener-specific intake levels were limited to congeners for which toxicological data was available (e.g., PCB126 for cancer or immune effects). Where toxicological studies dosed with a specific, defined mixture of PCBs (such as PCB101 and 118), they were compared against the sum of the estimated intake rates for each individual congener. Where toxicological studies dosed with an Aroclor or other commercial mixture of PCBs, they were compared against the estimated intake rate of total PCBs, unless otherwise noted.

In some cases, measurements of tissue concentrations reported a single concentration for two “coeluting” congeners. This meant that two congeners (e.g., PCB153/168) eluted from the gas chromatograph together, their identities could not be separated, and the reported concentration represented the sum of the coeluting congeners. PCB intakes of coeluted congeners were calculated in the same manner as non-coeluted congeners, as described above. However, because congeners reported as coelutes varied between samples/surveys, some congeners of interest had both individual and coelution measurements, and some congeners of interest were only measured in coelutions. A consistent approach was established to determine the appropriate intake rate for congeners of interest with reported concentrations in at least one coelution:

1. For congeners reported only as coelutions (e.g., PCB153 is not measured as a single elute, but is reported as PCB153/168), the intake rate of the congener of interest was conservatively assumed to be equivalent to the coelution concentration (i.e., the intake based on measurements of a PCB153/168 coelution was assumed to be 100% PCB153).
2. For congeners reported both as a single elute and as a coelute (e.g., PCB183 is measured both as a single elute and as a PCB183/185 coelution), or as a coelute in multiple coelutions, the greater of the resulting intake rates was applied to the congener of interest.

A summary of the approach used is provided in Table 4, which provides the fish consumption rates, PCB concentrations in raw fish tissue (for both PCB126 and total PCBs),⁶ and intake rates for PCB126 and total PCBs. Table 4 provides overall intake rates four ways:

- Method I calculated overall intake as the sum of products of the mean FCR and mean tissue concentrations for each fish species. This represents the best estimate of daily PCB intake from the Spokane River.
- Method II calculated the overall intake as the sum of products of mean FCR and 95th percentile tissue concentrations for each fish species.
- Method III calculated overall intake as the sum of the products of the 95th percentile FCR and mean tissue concentrations for each species. This method conservatively estimated the reasonable upper bound daily intake for consumers of Spokane River fish (see below).
- Method IV calculated overall intake as the sum of the products of the 95th percentile FCR and 95th percentile tissue concentrations for each species. This combination represents a hypothetical scenario bounded by the highest percentiles of each category.⁷

Methods II and III represent the reasonable upper bounds for intake from Spokane River fish consumption. Because intake rates from method III were greater than those associated with method II, method III intake rates were conservatively estimated as the reasonable upper bound daily congener-specific intake rates for each PCB congener measured and detected in fish samples (see Appendix 2).

⁶ No correction was applied to loss of PCBs that occurs upon cooking even though the process of cooking fish reduces median total PCB concentrations from 25 to 39% (AECOM, 2012).

⁷ The combined 95%-ile for both FCR and 95%-ile for fish tissue concentration is a hypothetical/impossible scenario for a consumer because it corresponds to (1) a lifetime of consuming fish at extremely high intake rates, (2) a lifetime of daily consumption of only fish with only the highest PCB levels, and (3) concentrations associated with raw fish tissues (because no correction factor was included to account for loss of PCBs that occurs during the process of cooking fish).

Table 4. Fish consumption rates, PCB concentrations, and intake from Spokane River fish consumption

Species	Number of Samples	Fish consumption rate (FCR; g/day)		PCB126 concentration* (ng/g)		Total PCBs concentration* (ng/g)	
		Mean	95 th percentile	Mean	95 th percentile	Mean	95 th percentile
Largemouth bass [+ Walleye]	6	2.56	11.2	0.0101	0.0137	68.3	98.4
Rainbow trout [+ Kokanee]	30	1.72	9.63	0.0108	0.0265	36.4	85.0
Mountain whitefish [+ Other]	12	0.10	0.0 [§]	0.0142	0.0216	118	189
Overall Method I (daily intake from all species data) (sum of products of mean FCR and mean tissue conc.)				0.0459 ng/day		249 ng/day	
Overall Method II (daily intake from all species data) (sum of products of mean FCR and 95 th percentile tissue conc.)				0.0827 ng/day		417 ng/day	
Overall Method III (daily intake from all species data) (sum of products of 95 th percentile FCR and mean tissue conc.)				0.217 ng/day		1,115 ng/day	
Overall Method IV (daily intake from all species data)[§] (sum of products of 95 th percentile FCR and 95 th percentile tissue conc.)				0.408 ng/day		1,919 ng/day	

* No correction was applied to loss of PCBs that occurs upon cooking even though the process of cooking fish reduces median total PCB concentrations from 25 to 39% (AECOM, 2012).

* Total values represent mean and 95th percentile for tissue concentrations for all fish species for which there was data.

[§]The combined 95%-ile for both FCR and 95%-ile for fish tissue concentration is a hypothetical/impossible scenario for a consumer because it corresponds to (1) a lifetime of consuming fish at extremely high intake rates, (2) a lifetime of daily consumption of only fish with only the highest PCB levels, and (3) concentrations associated with raw fish tissues (because no correction factor was included to account for loss of PCBs that occurs during the process of cooking fish).

B. Assessment of potential cancer risk from PCBs in fish in the Spokane River (see Appendix 3 for supplementary materials)

Sub-opinion 1: At the levels of PCBs measured in fish in the Spokane River, consumption of fish harvested from the Spokane River does not present any increase in cancer risk, even if consumed for a lifetime.

1. Introduction to cancer risk assessment

For chemicals that cause cancer in laboratory animals by creating damage to genetic material (DNA), many regulatory agencies use a different approach for assessing safety (or an 'acceptable level of risk'). This approach is called the 'linear, non-threshold' (LNT) approach, and basically assumes that cancer risk is directly proportional to dose at ALL doses (linear at low doses) below the point of departure determined in 2-year animal bioassays. This approach, which has dominated regulatory approaches to cancer risk assessment for many years, was used by the EPA in 1996 to establish guidance values for determining the level of PCBs in fish that do not represent an 'unreasonable risk' to human health. This approach was also used in the Washington Department of Health/Agency for Toxic Substances and Disease Registry (ATSDR) 2011 guidance document used to establish Fish Advisories and regulatory guidance on PCBs in Spokane River waterways and fish (WA Department of Health, 2011). However, in the past 20+ years, there have been enormous improvements in our scientific understanding of how chemicals cause adverse effects, at the molecular level.

The EPA and other regulatory agencies now embrace the inclusion of ‘mechanism’ or ‘mode of action’ (MoA) data into the risk assessment process⁸, especially in extrapolating adverse effects seen in animals at high doses to human exposures at doses many orders of magnitude lower. This is particularly important for PCBs because of the tremendous growth in our scientific understanding of the mode of action of PCBs in causing the adverse effects, including cancer, seen in laboratory animals given high doses of PCBs for a lifetime.

As my expertise is in toxicology, I have limited this evaluation to toxicological evaluation of animal studies that address the potential carcinogenic potential of PCBs found in fish. I have not done a detailed evaluation of the epidemiological literature, but have considered the opinions of Dr. Peter Shields, an expert in cancer epidemiology who has carefully evaluated the strengths and limitations of the epidemiological literature that address PCB exposures and potential cancer risk.

2. Hazard identification

Evidence from experimental animal studies that PCBs are able to reproducibly cause cancer in laboratory animals.

The first rodent bioassay for cancer was completed in 1971, with largely negative results. Subsequent studies using different strains of laboratory animals have had varying results, as shown in Table 5. Of 27 studies using several different commercial PCB mixtures, 6 of these reported statistically significant increases in tumors, including some malignant tumors (cancer), when comparing treated animals with controls. Of those 6 ‘positive’ studies, 5 were with Aroclor 1260 (or its equivalent, in the case of Kanechlor 500 or Clophen A60), and 1 (Brunner/Mayes) was with Aroclor 1254. The NCI study of Aroclor 1254 in 1978 was statistically ‘negative’ and although the report contained a caveat about the presence of preneoplastic lesions, the NCI concluded “Aroclor 1254 was not carcinogenic to the rats under the test conditions” (National Institutes of Health, 1978). Appendix 3 provides a detailed analysis of the results and conclusions of each of these studies.

In follow-up publications of findings from the NCI study, Morgan *et al.* (1981) and Ward (1985) reported a dose-related increase in the presence of metaplasia of the forestomach, and a non-statistically significant increase in a form of stomach cancer (adenocarcinoma) in several Aroclor 1254-treated animals. However, no other studies of Aroclor mixtures have reported this particular type of cancer. A slight, non-dose-related, increase in the incidence of thyroid gland follicular cell adenomas (benign tumors) was observed in males receiving Aroclors 1242, 1254, and 1260, but these findings have not been replicated in other studies.

⁸ “As understanding of mode of action improves and new types of data become available, dose-response assessment will continue to evolve. These cancer guidelines encourage the development and application of new methods that improve dose-response assessment by reflecting new scientific understanding and new sources of information” US EPA (2005). Guidelines for Carcinogen Risk Assessment. EPA/630/P-03/001F. March.

Table 5. Summary of 2-year carcinogenicity bioassays completed on commercial mixtures of PCBs

Year	Species and strain	Test group numbers	Dose groups (ppm)	PCBs studied	Positive for cancer (M, F)*	Reference
1971	CR Albino rats; males and females	8 groups; 50 per group	0, 1, 10, 100	Aroclor 1242	No, No	(IBT, 1971a)
				Aroclor 1254	No, No	(IBT, 1971b)
				Aroclor 1260	No, No	(IBT, 1971c)
1972	Dd mice	4 groups; 6-12 per group	0, 100, 250, 500	Kanechlor 300	No	(Nagasaki <i>et al.</i> , 1972)
				Kanechlor 400	No	(Ito <i>et al.</i> , 1973a; Ito <i>et al.</i> , 1973b)
				Kanechlor 500	Yes	
1974	Male Wistar rats	4 groups, 10-25 per group; fed for 1 yr	0, 100, 500, 1000	Kanechlor 300	No	(Ito <i>et al.</i> , 1974)
				Kanechlor 400	No	
				Kanechlor 500	No	
1975	Female Sherman rats	2 groups, 200 per group	0, 100	Aroclor 1260	Yes	(Kimbrough <i>et al.</i> , 1975)
1978	Male and Female F344 rats	8 groups; 24 per group	0, 25, 50, 100	Aroclor 1254	No, No ^{*9}	(Moore <i>et al.</i> , 1994; Morgan <i>et al.</i> , 1981; NCI, 1978; Ward, 1985)
1984	Male Wistar rats	3 groups; 131 in control 138 in A30 129 in A60	0, 100	Clophen A30	No	(Schaeffer <i>et al.</i> , 1984)
				Clophen A60	Yes	
1985	Male and Female Sprague-Dawley rats	6 groups; 63-70 per group	0, 100 for 16 mo + 50 for 8 mo	Aroclor 1260	No (M) Yes (F)	(Norback and Weltman, 1985)
1998	Male and Female Sprague-Dawley rats	30 groups; 50 animals per group	0, 50, 100, 200	Aroclor 1016	No (M)** No (F)**	(Brunner <i>et al.</i> , 1997; Mayes <i>et al.</i> , 1998)
			0, 50, 100	Aroclor 1242	No (M) No (F)	
			0, 25, 50, 100	Aroclor 1254 ¹⁰	No (M) Yes (F)	
			0, 25, 50, 100	Aroclor 1260	No (M) Yes (F)	
			0, 25, 50, 100			

* The results shown in this column represent studies that identified malignant tumors (cancers). Some studies found increased numbers of non-malignant liver tumors, called hepatocellular adenomas.

** In the Mayes *et al.* (1998) study, one hepatocellular carcinoma was observed in the 100 ppm female group, but none were seen at the higher dose (200 ppm). No carcinomas were seen in the female control. This finding was not statistically significantly different from control, although several adenomas were seen in the 100 and 200 ppm Aroclor 1016 group that did reach statistical significance. In males exposed to 1016, 2 hepatocellular carcinomas were seen in the high dose treatment, and 1 each in the two lower dose groups, but there were 3 in the controls, so it was concluded that the carcinomas seen in 1016-treated males were not treatment related.

Because the US EPA had begun using these studies to establish regulatory guidelines for all PCBs, the National Toxicology Program (NTP) organized a "Pathology Working Group" (PWG) consisting of 5 independent, Board certified Veterinary Pathologists to review the original histopathology (microscopic evaluation of tissues from each of the animals) from 4 data sets containing 7 different bioassays (Kimbrough *et al.*, 1975; NCI, 1978; Norback and Weltman, 1985; Schaeffer *et al.*, 1984). The consensus report was published by Moore *et al.* (1994). The reevaluation of the pathology of animals in the seven previous rat bioassays found relatively modest differences from the original reports, with a few exceptions (discussed in Appendix 3). The conclusions of the working group were:

"The reevaluations permit a more confident comparison of the carcinogenic responses observed in seven different studies with PCBs. The results highlight three issues: PCBs with 60% chlorine content consistently provoke a high yield of liver tumors in rats; the liver tumor response observed in rats exposed to PCBs with lower levels of chlorine was not observed to have an increase in liver tumors; no clear sensitivity differences in tumor response were observed between males and females. These data indicate that continuation of a science policy of assuming that all PCBs are probable human carcinogens and possess a carcinogenic potency equivalent to the mixture that contains 60% chlorine has no scientific foundation and should be reconsidered."

However, the subsequent extensive bioassays of different Aroclor mixtures by Brunner *et al.* (1997) reported in peer reviewed literature as Mayes *et al.* (1998) demonstrated that Aroclor 1254, like 1260, also produces liver tumors in female Sprague-Dawley rats. It is important to note, however, that the 'positive' Brunner/Mayes study utilized a formulation of Aroclor 1254 that was manufactured by a different process than had been used prior to 1974 and had considerably higher amounts of DL-PCBs, compared to 'older' Aroclor 1254 formulations used in the NCI 1977 study and the 1971 IBT study (Kodavanti *et al.*, 2001). The DL-PCB 'TEQ' value for the Aroclor 1254 lot used in the Brunner/ Mayes study was 11 times higher than the TEQ value of Aroclor 1254 that was used in the largely 'negative' NCI 1978 and IBT 1971 studies (Kodavanti *et al.*, 2001), providing further support for the now widely recognized fact that the hepatocarcinogenic effects of Aroclor mixtures seen in rats are due almost completely to the presence of DL-PCBs. At high doses that cause substantial microsomal enzyme induction, non-DL PCBs will contribute to oxidative stress which, over the lifespan of the animals contribute to the development of adenomas and possibly carcinomas. But doses of non-DL PCBs needed to do this are extremely high. For doses of PCB mixtures that contain both DL and Non-DL PCB congeners (e.g., Aroclors or environmental sources such as fish), the induction of AhR by DL-PCBs is much more sensitive than induction of CAR and PXR-regulated microsomal enzymes. Thus, at doses of PCB mixtures that do not induce AhR activity, it is highly unlikely that biologically significant induction of CAR and PXR would occur.

⁹ The conclusions of the 1978 Bioassay of Aroclor 1254 states the following: *"It is concluded that under the conditions of this bioassay, Aroclor® 1254 was not carcinogenic in Fischer 344 rats; however, a high incidence of hepatocellular proliferative lesions in both male and female rats was related to treatment. In addition, the carcinomas of the gastrointestinal tract may be associated with treatment in both males and females"* (NCI, 1978).

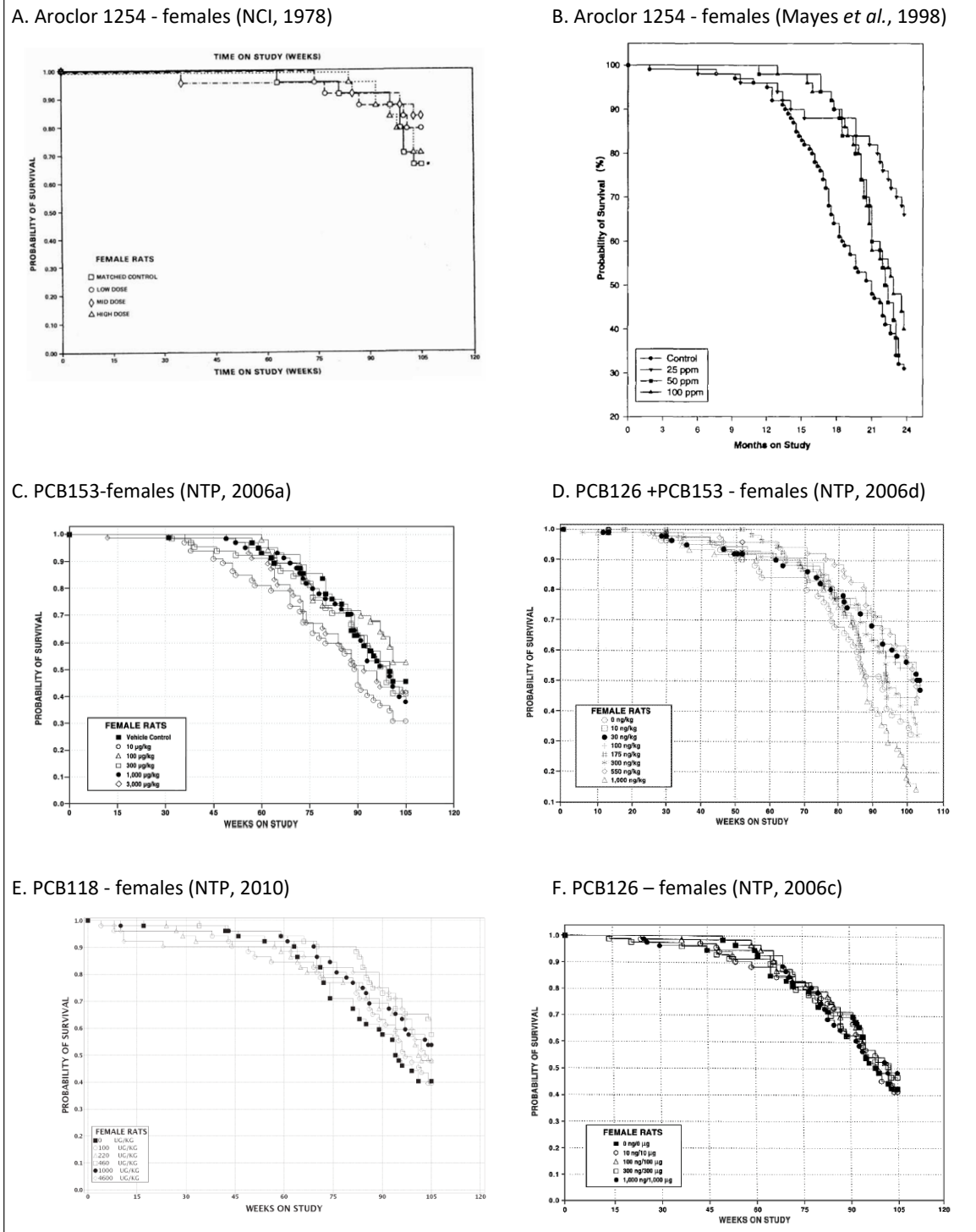
¹⁰ The Mayes *et al.* (1998) study used an Aroclor 1254 formulation that contained much higher levels of dioxin-like PCBs than previous studies of Aroclor 1254 (Kodavanti *et al.*, 2001).

Finally, as discussed in detail in the 'Mode of Action' and 'Dose-Response Modelling' sections below, beginning in 2006, the National Toxicology Program reported on a series of 2 year rat bioassays on specific PCB congeners, including two DL-PCBs, PCB126 (NTP, 2006c) and PCB118 (NTP, 2010), one 'non-DL-PCB, PCB153 (NTP, 2006a), combinations of PCB126 with PCB118 or PCB153 (NTP, 2006d, e), and 'dioxin' itself (2,3,7,8-Tetrachlorodibenzodioxin; TCDD) (NTP, 2006b). The results from these studies are discussed in detail in Appendix 3 and below. The overall conclusions from these mechanistic studies support the conclusions above that PCB mixtures containing 'dioxin-like' congeners (e.g., PCB126 and PCB118) increase the production of liver tumors in female rats, but also support the conclusion that PCBs with little or no 'dioxin-like' activity are, at most, weak promoters of liver cancer in rats, since PCB153 did not cause a statistically significant increase in liver tumors in male or female rats (See Appendix 3 for details). However, it is possible that the presence of non-DL PCBs (such as PCB153), at doses sufficient to cause significant induction of CAR and/or PXR in the rat, might enhance the carcinogenic response when combined with DL-PCBs that cause maximal, prolonged activation of AhR. This was seen in the NTP studies of a combination of PCB126 and PCB153 where the number of tumors in the combined treatment group was greater than those seen with PCB126 alone. However, when PCB153 was present at a dose (100 µg/kg) that caused only modest induction of microsomal enzymes, there was no effect of PCB153 on the incidence of either benign (adenomas) or malignant (choriocarcinomas) tumors in female rats given 300 ng/kg PCB126 (NTP, 2006a, c, d).

In all of these studies, liver cancer was the only consistent type of cancer found. Although an increase in incidence of some other tumor types was found in these studies, overall tumor incidence in many of these studies was decreased, because of the marked protective effect that PCBs provided in some types of tumors, particularly breast tumors in female rats (Moore *et al.*, 1994). It is also important to note that in none of the studies was life expectancy shortened by the presence of tumors (i.e., while the number of animals with tumors was increased at the highest dose, the tumors were found in the animals at the end of the study, and the animals did not generally die from the tumors). In fact, in some studies, such as the NTP study on PCB118 (Figure 8B) there was a dose-related increase in survival at 2 years, compared to control animals, with little or no effect on overall survival in most others (Figure 8A-D).

Appendix 3 provides a detailed review of the findings of each of these studies. Using a weight of evidence approach to assess these studies, it can be concluded that there is convincing evidence that higher chlorinated PCB mixtures (Aroclor 1254 and 1260) are capable of inducing hepatic cancers in some strains of rats, and particularly in female rats. Elsewhere in this report I explain that the levels of PCBs administered in these studies are many hundreds of times higher than human doses and that rats are far more sensitive to AhR- binding than humans. For these and other reasons, the positive results from these studies do not predict the development of cancers in humans.

Figure 8. Kaplan-Meier survival curves for several Aroclor/PCB studies



What approach did the US EPA use to estimate cancer risk from exposures to PCBs that serves as the basis for current environmental regulations for PCB discharges into the Spokane River?

Nearly all regulatory authorities in the US, be it city, county, state or other federal agencies, rely upon the US Environmental Protection Agency's (US EPA) risk assessments of environmental pollutants such as PCBs to establish regulatory guidelines and values. This is especially true for chemicals found in the environment that have been shown to cause cancer in laboratory animals and are subject to regulatory restrictions. The EPA completed a detailed 'cancer risk assessment' for PCBs in 1996 (US EPA, 1996). They evaluated all of the existing life-time (2-year) animal studies and calculated 'human potency factors' for each study (Table 6), using a variety of assumptions to be addressed later (See Appendix 3 for a detailed review of these and more recent animal studies).

Using additional assumptions, the EPA chose a single 'potency factor' of 2 (per md/kg-d), based on the Brunner *et al.* (1997) study of Aroclor 1254-induced liver tumors in female rats, the Norback and Weltman 1985 study of liver tumors in female rats exposed to Aroclor 1260, and the Schaeffer study of Clophen A60 [Aroclor 1260-like] liver tumors in rats (Table 6). This was the 'upper bound' of the slope factor that gave the highest level of predicted risk (most cancers) from all of the mentioned rat studies (rounded to a whole number), **assuming linear extrapolation at low doses**. This single potency value, based on the incidence of rat liver tumors in female rats, continues to serve as the sole basis for quantitative cancer risk assessment of PCBs. Since the vast majority of human health risk assessments for PCBs use cancer as the most sensitive endpoint, cancer risk has been the 'driver' for regulatory guidance for PCBs since 1996. The 'cancer potency factor' is used to determine regulatory limits, including effluent discharge levels, by the Washington State Department of Ecology and the US EPA in their derivation of human health criteria for water quality standards for PCBs in surface waters (US EPA, 2016, 2019a, b)

Table 6. Human potency and slope estimates derived from rat liver tumors (from: US EPA, 1996)

<u>Study, sex and strain, mixture</u>	<u>ED10^a</u>	<u>LED10^b</u>	<u>Central slope^c</u>	<u>Upper-bound slope^d</u>	<u>See table</u>
Brunner, F Sprague-Dawley, 1260	0.24	0.19	0.4	0.5	A-1
Brunner, F Sprague-Dawley, 1254	0.086	0.067	1.2	1.5	A-2
Brunner, F Sprague-Dawley, 1242	0.38	0.27	0.3	0.4	A-3
Brunner, F Sprague-Dawley, 1016	2.4	1.4	0.04	0.07	A-4
Brunner, M Sprague-Dawley, 1260	1.0	0.55	0.1	0.2	A-5
Brunner, M Sprague-Dawley, 1254 ^e	1.7	0.87	0.06	0.1	A-6
Brunner, M Sprague-Dawley, 1242 ^e	2.9	1.2	0.03	0.08	A-7
Brunner, M Sprague-Dawley, 1016 ^e	5.9	2.5	0.02	0.04	A-8
Kimbrough, F Sherman, 1260	0.10	0.091	1.0	1.1	A-9
NCI, M Fischer, 1254	1.0	0.55	0.1	0.2	A-10
NCI, F Fischer, 1254 ^e	1.2	0.61	0.08	0.2	A-11
Schaeffer, M Wistar, A 30 ^e	2.1	1.0	0.05	0.1	A-12
Schaeffer, M Wistar, A 60	0.058	0.047	1.7	2.1	A-13
Norback, M Sprague-Dawley, 1260 ^e	1.0	0.53	0.1	0.2	A-14
Norback, F Sprague-Dawley, 1260	0.062	0.046	1.6	2.2	A-15

^aEstimated dose associated with 10% increased incidence, in mg/kg-d.
^b95% lower bound on ED10, in mg/kg-d.
^cPer mg/kg-d, computed as 0.10/ED10.
^dPer mg/kg-d, computed as 0.10/LED10.
^eNo significant increase; quantities indicate sensitivity of study.

At the time that the USEPA established these cancer potency estimates (US EPA, 1996) from rat studies, the mode of action of PCBs in causing the liver tumors seen in these studies was not understood, although it was recognized that higher doses of PCBs could cause dramatic changes in the liver, including extensive 'microsomal enzyme induction.' The phrase, 'microsomal enzyme induction' has an important meaning in toxicology, as it represents what is now recognized as a part of an adaptive response to environmental insult. Microsomal enzymes are a group of proteins, found mostly in the liver, that play an important role in the body's defense against external influences, such as exposure to potentially toxic substances in the environment. The importance of microsomal enzyme induction to the understanding of the toxic effects of PCBs will be discussed in more detail in the 'mode of action' section below.

It is important to note that the animal studies used by the EPA and other regulatory agencies to establish health-based standards for PCBs in the 1990's do not represent the current state of scientific understanding, including the EPA's current understanding, of the potential cancer-causing effects of PCBs in laboratory animals. Since that time, additional state-of-the-art animal bioassays have been completed, including the series of studies, noted above, of individual congeners by the National Toxicology Program (NTP; part of the National Institute of Environmental Health Sciences, NIEHS). These studies confirmed some of the initial findings in earlier studies used by the EPA (primarily, liver tumors in female rats), but provided much more robust, scientifically useful data from which to extrapolate the results to humans. The results of these carefully designed studies call into question many of the assumptions used by the EPA in their 1996 assessment and provide a much better understanding of the 'mode of action' of how PCBs cause liver tumors in female rats, and how one might appropriately use such information to better predict the relevance of these animal studies to human cancer risk from PCB exposure.

Collectively, the animal studies conducted between 1972 and 1996 demonstrate that higher chlorinated mixtures of PCBs, such as Aroclor 1254 and 1260, are able to induce liver tumors in rats. Later studies on individual PCB congeners conducted by the National Toxicology Program (NTP, 2006a, c, d, e, 2010) provided further evidence that PCB mixtures containing significant amounts of DL-PCBs are effective carcinogens in laboratory rats. However, this response appears to be greatly influenced by both sex (females being much more sensitive than males) and by rat strain (Sprague-Dawley and Wistar rats being much more sensitive than Fischer F344 rats). Appendix 3 provides a detailed discussion of all of these studies.

Thus, a potential cancer hazard has been identified based on studies in laboratory rats, and requires further analysis to assess the relevance to humans, and the potential magnitude of risk under specific exposure conditions (e.g., a human 'dose-response' analysis, comparing human doses with the doses shown to cause effects in laboratory animals). The next step after Hazard Identification is to evaluate how PCBs might have caused the excess liver tumors in female rats that has been demonstrated in several animal studies, and that serve as the basis for the US EPA's quantitative cancer risk assessments— what is the 'Mode of Action' for the observed carcinogenic effects in some strains of laboratory rats, and what does that Mode of Action tell us about the likely shape of the dose-response curve at doses orders of magnitude below the doses that caused tumors in rats?

3. Mode of action for PCB-induced carcinogenesis in rats

As shown above, there are several animal studies that have shown that PCBs can cause liver tumors, including some that are cancerous (malignant) in laboratory rats, especially females. So, once a 'hazard' (in this case cancer-causing effects observed at high doses in experimental animals) has been established, the next step in the Risk Assessment paradigm is to assess what is known about 'how' the chemical causes the effect: what are the cellular, biochemical and molecular steps involved that result in a normal cell in the body (the liver, in this case) turning into a cancer cell? In the field of chemical carcinogenesis, there are three broad processes in the development of cancer: 1) initiation (genetic changes that turn a 'normal cell' into a 'precancerous cell'), 2) promotion (the precancerous cell is stimulated to divide into a small population of precancerous cells) and 3) progression (additional genetic changes are acquired that allow the precancerous cells to acquire additional characteristics of cancer cells, including spreading to other tissues [metastasis]) (Figure 9).

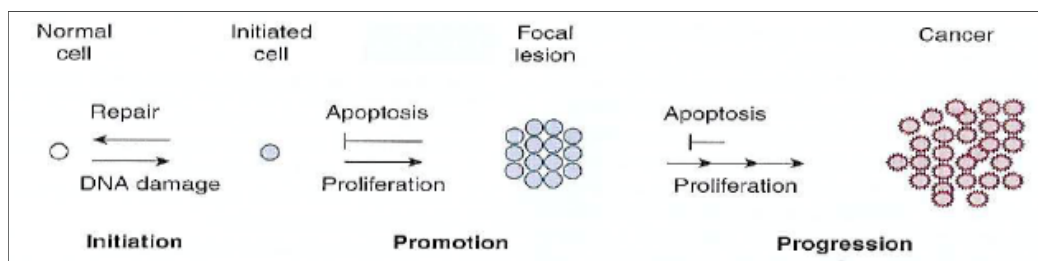


Figure 9. The multistage process of chemical carcinogenesis (from: Klaunig, 2013)

While this three-stage process is a gross over-simplification of very complicated biology, it is a useful paradigm to start with, since it is well-ingrained in regulatory philosophy of chemical carcinogens (Eaton and Gilbert, 2013; Faustman and Omenn, 2013; Klaunig, 2013). Chemicals that can cause the initial genetic changes are called "Initiators", and chemicals that can enhance the growth of initiated cells (in both the 'promotion' and 'progression' stages) are called "Promoters." In the following section I will discuss both 'Initiation' and 'Promotion' in relation to how PCBs caused liver cancer in laboratory rats, and relevance to human cancer risk from dietary exposures to PCBs.

Initiation (Genotoxic mode of action)

Simply put, 'initiators' are chemicals that cause cancers by directly damaging DNA (and thus are referred to as 'genotoxic carcinogens'), thereby inducing mutations, which can be passed on from one cell to the next during cell division. Loeb and Harris (Loeb and Harris, 2008), in their discussion of the history of the field of chemical carcinogenesis, describe this first step in cancer formation as follows: *"The first stage of carcinogenesis, tumor initiation, involves exposure of normal cells to chemical or physical carcinogens. These carcinogens cause genetic damage to DNA and other cellular macromolecules that provide initiated cells with both an altered responsiveness to their microenvironment and a proliferative advantage relative to the surrounding normal cells."* It is generally recognized that the process of going from a 'normal cell' to a cancer cell requires multiple mutations in different genes. Mutations must 'turn on' certain genes, called 'oncogenes', that are normally turned off, AND they also must 'turn off' certain other genes, called 'Tumor Suppressor genes' that function to control the rate of cell division in cells. Subsequent additional mutations

are needed that allow precancerous cells (those with activated oncogenes and inhibited tumor suppressor genes) to become full-blown cancers. It has been estimated that most cancer require 4-10 or more mutations for a normal cell to become a cancer cell (Martincorena *et al.*, 2017).

Because mutagenesis is so important to the process of chemical carcinogenesis (formation of cancers from normal cells), there are a large number of *in vitro* and *in vivo* tests in animal models to determine if a chemical might cause cancer by inducing excess mutations – e.g., act as a genotoxic carcinogen. Not surprisingly, these tests are subject to numerous experimental artifacts and interpretive challenges, and can result in both ‘false negatives’ (a substance tests negative, even though it actually is capable of causing DNA damage) and ‘false positive’ (a substance tests positive in the test system, but is not carcinogenic via damaging DNA). There are multiple different ways a chemical can damage DNA: 1) it can directly interact with and bind to DNA, disrupting normal functions of DNA and introduce mutations; 2) it can be metabolized in the body to a reactive form that can then interact with and bind to DNA, disrupting normal functions of DNA and introducing mutations; 3) it can directly or indirectly generate high levels of ‘oxidative stress’, which itself can cause DNA damage that disrupts normal functions of DNA and introduce mutations; or 4) it can interfere with normal DNA repair processes, allowing ‘background DNA damage’ to go unrepaired.

Hundreds of studies have addressed whether PCBs are genotoxic and can increase cancer risk via damaging DNA. Because of the large number of studies that have looked at this question, it is necessary to take a ‘weight of evidence’ approach to assess whether this mode of action is a plausible explanation for the induction of liver tumors seen in laboratory rats that serves as the basis for the EPA’s cancer potency values (slope factors) that were used by WDOH and ATSDR to evaluate theoretical cancer risk from PCBs in Spokane River fish. Table A3-1 and Table A3-2 in Appendix 3 provides a summary of the large number of ‘short-term’ mutagenicity assays that have been performed on mixtures of PCBs, individual PCB congeners, and synthetic metabolites (artificially oxidized) metabolites of PCBs.

The most obvious conclusion that is evident from a ‘weight of evidence’ evaluation of these data is that the results are highly variable across test types, PCB mixtures, and individual congeners. Nevertheless, several important conclusions can be made, based on the weight of evidence:

1. The large majority of mutagenicity assays for Aroclors and other mixtures of PCBs are negative;
2. There is modest consistent evidence that lower chlorinated PCBs, largely focused on monochlorobiphenyls (e.g., PCB3) are capable of being oxidized to reactive forms that may be mutagenic, but these forms do not bioaccumulate extensively and thus are not found in significant quantities in fish;
3. Some putative positive studies in mammalian cells are difficult to interpret because of many uncontrollable variables, uncertainty as to relevance of ‘dose’ in a cell culture flask compared to human blood/tissue levels, and inconsistency across different studies.

Knerr and Schrenk (2006) reviewed the mutagenesis data on PCBs, and the potential relevance to their carcinogen risk, and provided the following conclusions: “Since most tests with PCBs, in particular with *in vivo* systems, for genotoxic effects were negative, the carcinogenicity of PCBs in animal models is widely considered to be due to a tumor-promoting effect.”

It is important to recognize that damage to DNA that potentially can lead to cancer-causing mutations is a common occurrence, largely from endogenous (naturally-occurring) sources. The most common source of DNA damage is from intracellular oxidative processes that generate forms of reactive oxygen, known as 'ROS' (reactive oxygen species). While many chemicals present in our diet and the environment have the potential to damage DNA, either directly, or via induction of ROS, the vast majority of this DNA damage is repaired before the cell divides, and thus does not cause a mutation if the damage is repaired correctly (Jackson and Loeb, 2001). The relevance of this to my opinion is that the level of DNA damage that could theoretically occur via oxidation of some forms of PCBs present in fish at low part per billion concentrations, compared to 'background' rates of oxidative damage to DNA, is vanishingly small, and would not contribute in any significant way to the rate of background DNA damage and mutations that occur from other sources (largely 'endogenous'). Given the preponderance of the data showing little or no mutagenic potential of PCB congeners found in fish, and the questionable relevance of most of the positive studies, when combined with the extremely small amounts of PCBs that come from ingesting fish from the Spokane River, **it is my opinion that the hypothetical mutagenic action of the mixture of PCB congeners found in fish in the Spokane River and other aquatic environments, if any, is so low as to be insignificant compared to the background rate of mutations than happens naturally.**

Tumor promotion as a general mode of action for the carcinogenic action of PCBs in laboratory rats

- **Sub-Opinion: There is clear evidence that the mode of action of PCBs in causing liver cancer in laboratory rats exposed to high doses of PCBs is via tumor promotion**

The term 'tumor promotion' refers to the circumstance where a chemical substance increases the number of tumors that appear in an exposed population of animals, but without evidence of direct genetic damage from the agent. Loeb and Harris (2008) describe tumor promotion as follows: *"In the second stage, tumor promotion results in proliferation of the initiated cells to a greater extent than normal cells and enhances the probability of additional genetic damage, including endogenous mutations that accumulate in the expanding population"*, but they caution that *"This classic view of two- stage carcinogenesis has been conceptually important but also an oversimplification of our increasing understanding of the multiplicity of biological processes that are deregulated in cancer."*

From a regulatory perspective, the designation of a chemical as either a 'tumor initiator' or a 'tumor promoter' has significant implications, since the EPA and most other regulatory agencies have traditionally assumed that initiators have a 'linear, non-threshold' dose-response, whereas promoters are considered to act through 'threshold' mechanisms – e.g., there is a dose below which no effect will occur (Klaunig, 2013). The processes by which tumor promotion can occur are varied and many, but generally include acting via some mechanism(s) that enhances the rate of cell proliferation and/or inhibit normal processes that remove cells with genetic damage (called 'apoptosis'). This includes agents that cause organ system toxicity that results in an adaptive response of tissue repair. Thus, at doses that are sufficient to cause evidence of liver toxicity, regenerative repair of the liver leads to increased cell division, and this by itself can act as a form of 'tumor promotion.' Chemical-induced changes in a cell, such as receptor-mediated changes in gene expression (e.g., ligand activation of AhR, CAR, PXR, etc.) that can result in an increase in 'oxidative stress' and/or abnormal gene expression of genes involved in cell cycle regulation are other means by which a chemical could act as a 'tumor promoter.' Typical experimental protocols for determining whether a chemical can act as a tumor promoter involves administering a high dose of a genotoxic chemical (an

‘initiator’) over a short period of time (one to a few days), followed by administration of the ‘promoter’ for longer periods of time (usually several months). Typically, the time period and dose of the ‘initiator’ are not sufficient to cause a large increase in tumors by itself. But in the presence of a ‘promoter’, the number of animals with tumors (and, sometimes, the number of tumors in individual animals) is greatly increased – thus, tumor development and growth are ‘promoted.’

There are two dozen studies that have demonstrated that PCB mixtures are capable of promoting tumor development following initiation with known genotoxic carcinogens (reviewed in Knerr and Schrenk (2006)). Thus, there is no question that PCBs in sufficient dose and length of time of exposure are capable of acting as tumor promoters in laboratory animals. The remaining questions are: 1) what are the specific molecular events that lead to tumor promotion in laboratory animals and are they relevant to humans?; 2) what doses are required for such molecular changes to occur in experimental animals?; and 3) if the specific mechanisms are applicable to humans, what is the ‘Margin of Exposure’ between the doses necessary to cause the effect in animals, and the doses estimated for humans?

- **The ‘mechanism of action’ by which PCBs caused the tumors in laboratory animals is dependent upon extensive and prolonged activation of the ‘Aryl hydrocarbon Receptor’ (AhR)**

As discussed in the introductory section of this report, the toxic effects of many chemicals occur through their ability to interact with specific biological receptors in the body, producing toxic responses. The best studied of all ‘ligand-receptor interactions’ and the unwanted biological responses that occur is that of dioxins and dioxin-like compounds interacting with the Aryl hydrocarbon Receptor (AhR). As discussed in the introduction, there are 12 forms (congeners) among the 209 possible PCB molecules that can interact with and activate the rodent (rat and mouse) AhR. These 12 forms are known as Dioxin-Like PCBs (DL-PCBs). Although all 12 forms have some ability to activate the rodent AhR, they differ vastly in their potency. This important point will be discussed in detail later.

Once a dose of activating ligand such as dioxin, or a DL-PCB, is high enough to activate a large number of AhRs, a series of biological events occur that may ultimately result in unwanted, toxic effects, which can include cancer, at least in rodents. When a sufficient number of dioxin-like molecules bind to the AhR, additional molecules in the cell (called co-factors) attach, and the complex is transported into the nucleus of a cell, where it recognizes specific regions of DNA, called ‘Dioxin Response Elements (DREs) (Figure 10).

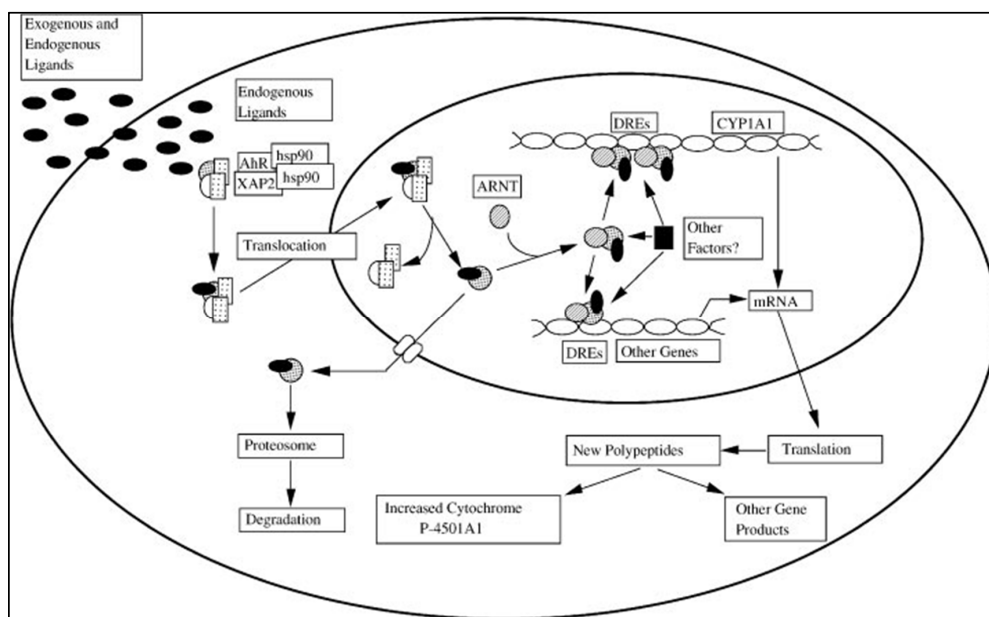


Figure 10. The molecular mechanism of activation of gene expression by the AhR (from: Denison and Nagy, 2003)

When the AhR-ligand complex binds to these DREs in the cell nucleus, it turns on a set of specific genes, causing them to make sometimes large amounts of proteins that have specific biological functions. The magnitude of this response (how much new protein is made) is dependent on dose. At low doses that are insufficient to occupy a significant number of receptors, no response will be evident. But at high doses, many receptors are occupied, which triggers the increase in protein synthesis. “Dose” here refers to the combination of the amount of activating ligand, and its relative affinity for the receptor (i.e., potency). When a gene is activated the DNA code is ‘transcribed’ into an intermediate molecule called messenger RNA (mRNA), which in turn is ‘translated’ into a protein which has biological function. Thus, receptor complexes like the AhR, when bound to a ligand, are called ‘transcription factors’ since they cause specific genes to be transcribed into mRNA. It is the ‘over-expression’ of these genes that is ultimately responsible for the toxic effects observed from dioxins and dioxin-like molecules. If the dose/potency of the activating ligand is not sufficient to cause the overexpression of these genes, then no response will occur. Scientists often talk about ‘down-stream effects’ that result when a particular gene is activated by a transcription factor. Perhaps the best example of this is a specific gene called ‘Cytochrome P4501A1’ (CYP1A1). CYP1A1 is present in nearly all tissues, but usually at very low levels. However, when dioxin-like molecules are present in high enough concentrations, they bind to the AhR and induce the expression of CYP1A1 (Figure 10). This protein functions as a ‘drug metabolizing enzyme’ which can break down both exogenous (e.g., certain drugs and environmental chemicals) and endogenous (e.g., estrogen) molecules. There is strong evidence that much – but not all- of the toxic effects of dioxin-like molecules is due to the high levels of expression of CYP1A1 that happen after exposure to dioxin-like molecules. Like all toxic responses, the level of induction of CYP1A1 is dependent on both the amount of activating ligand (e.g., dioxin, DL-PCBs) AND the affinity of the ligand for the AhR (the potency of the ligand). As is typical with all receptor-ligand interactions, the slope of the dose-

response curve is very steep (see for example, Figure 5, Estrogen Receptor activation). At doses below the threshold of activation, there are no biological responses because the concentration of ligand at the receptor site is insufficient to activate enough receptors to produce a measurable biological response. However, once the dose reaches a concentration sufficient to begin activating the receptors, further increases in dose result in proportional increases in response, until the dose is sufficient to occupy nearly all the receptors. Once this occurs, the receptors are 'saturated' and higher doses do not produce an increase in effect. This will be illustrated later with specific data on some DL-PCBs that cause liver tumors in laboratory rats. (See Appendix 3 for a more detailed description of how the AhR functions to produce toxic responses in cells following activation by dioxin-like compounds.)

It is widely recognized that the toxicological effects of the DL-PCBs, including the production of tumors in rats, are dependent upon the relative abilities of different DL-PCB congeners to bind to and activate the AhR. Based on this widely recognized concept, every regulatory body in the world with authority for PCB regulations utilizes the concept of "Toxic Equivalency Factors", or TEFs, to assess the potential toxicity of PCB mixtures. Since the 1990s, the World Health Organization (WHO) has organized expert meetings to review the scientific basis for establishing the TEFs for dioxins and dioxin-like compounds at the international level, thus providing consistent approaches to risk assessment to national/international regulatory authorities (van den Berg *et al.*, 2006).

Numerous recent reviews have exhaustively described the sequence of events that occur following extensive activation of the AhR by dioxins and dioxin-like compounds (See Appendix 3 for details). The complex series of 'downstream' events that lead to changes in gene expression and associated biochemical changes result in a wide variety of adverse effects, including but not limited to tumors, immune toxicity, liver toxicity, chloracne and other dermatological effects, and adverse reproductive and developmental outcomes. Thus, all of these adverse outcomes are dependent upon the 'Key Initiating [Molecular] Event' of activation of the AhR. Therefore, to estimate the potential health risks from exposures to DL-PCBs it is only necessary to identify the dose-response relationship for the DL-PCBs that can activate the human AhR and determine a biological 'no effect' level in target tissues. Indeed, the EPA encourages the utilization of 'mode of action' data in conducting risk assessments. For example, in the 2005 Guidelines for Carcinogen Assessment, EPA states "*As understanding of mode of action improves and new types of data become available, dose-response assessment will continue to evolve. These cancer guidelines encourage the development and application of new methods that improve dose-response assessment by reflecting new scientific understanding and new sources of information.*" (US EPA, 2005, p. 3-2).

A more comprehensive discussion of how 'mode of action' data are currently being used in Risk Assessment via the 'Adverse Outcomes Pathways' approach to chemical risk assessment can be found in Appendix 3.

More recent animal studies have demonstrated that the AhR must be maximally activated for much of the lifespans of the animals to cause a significant increase in liver tumors. Many receptors in the body have significant biological effects even if they are only activated at 25 to 50% of the maximal possible amount, so relatively small additions to the normal activation that might come from an exogenous drug or environmental chemical might have important biological consequences. This is not the case for the cancer-causing effects of DL-PCBs in female rats, as shown by a series of elegant experiments conducted by the National Toxicology

Program (NTP). The NTP, a Congressionally-mandated division within the National Institute of Environmental Health Sciences (NIEHS), is widely recognized as the leading authority in the world in the conduct, evaluation and interpretation of 2-year (lifetime) laboratory animal bioassays to determine the carcinogenic potential of environmental contaminants. In the mid-2000's, the NTP conducted a series of detailed, 2-year bioassays on various specific congeners of PCBs, dioxins and dibenzofurans to determine if activation of the AhR is the sole mechanism by which dioxins and dioxin-like PCBs cause cancers in laboratory rats. In addition to assessing dioxin (TCDD) itself, they analyzed two dioxin-like PCBs (PCB126 and PCB118) and one 'non-dioxin like' PCB, PCB153, as well as some combinations of these congeners. [See Appendix 3B and C for detailed discussion of how activation of the AhR is the Key Molecular Event in the mode of action for liver cancer development following administration of dioxins and dioxin like compounds to laboratory rats, and a detailed analysis of the NTP studies on PCB126, PCB118, PCB153 and TCDD (NTP, 2006a, b, c, d, e, 2010)].

The finding that the AhR must be maximally activated for much of the lifespans of the animals to cause a significant increase in liver tumors is important because the 'Margins of Exposure' analysis used in this report identifies the point of departure where only minimal activation of the AhR occurs. This provides another 'implicit' Margin of Exposure of perhaps 1 order of magnitude (factor of 10), since adverse effects would not be anticipated until the dose was sufficient to produce a much greater level of AhR activation than would occur at the 'Point of Departure' chosen for calculating Margins of Exposure.

- **Consideration of an Alternative Mode of Action for liver tumor promotion by PCBs: Activation of nuclear receptors CAR and/or PXR**

As discussed above and in Appendix 3, there is extensive scientific evidence to demonstrate that activation of the AhR by DL-PCBs and subsequent downstream changes in gene expression in the liver is the primary mechanism by which PCB mixtures promote the development of liver tumors in laboratory animals. However, there is limited evidence that non-dioxin like PCBs may also act as liver promoters. For example, the (Mayes *et al.*, 1998) study on Aroclor 1016 found a small number of benign liver adenomas at the two highest doses (100 and 200 ppm), even though the dioxin 'TEQ' in the Aroclor 1016 mixture was quite low, relative to Aroclor 1254 and 1260. And some of the earlier studies on Aroclor 1260 found tumors at the highest dose groups, even though the DL-PCB content in Aroclor 1260 is not as high as Aroclor 1254. But there is substantial evidence that the mechanism of tumor promotion by NDL-PCBs is via changes in gene expression that is secondary to activation of nuclear receptors CAR and PXR, and results in altered metabolism of endogenous hormones and other biomolecules at high doses. A variety of NDL-PCBs have been shown to be effective agonists of both rat CAR and PXR (Gahrs *et al.*, 2013) (Discussed in Appendix 3). However, the significance of this to humans is questionable, since recent studies have shown that PCBs are not effective activating ligands for human PXR (Tabb *et al.*, 2004). Furthermore, the promotion of liver tumors in rats by activating CAR ligands such as phenobarbital, after extensive evaluation of existing data, have been shown to not be relevant to human liver cancer risk (reviewed in Appendix 3). A workshop with broad participation from many scientists from government (including EPA), academia and industry was held to address the question of the relevance of Nuclear Receptor activation (including AhR, CAR and PXR) as a mode of action for human liver cancer risk (Andersen *et al.*, 2013). The findings from that workshop report confirmed that the AhR mode of action was relevant to humans and recommended further analyses for dose-response modeling. The subsequent report of the workshop discussed above by Budinsky *et al.* (2014) discussed above presents the outcome for AhR mode of action and dose-response modeling for liver cancer

called for by the Andersen *et al.* consensus report (Andersen *et al.*, 2013). The sub-panel of 21 of the world's top experts on the molecular functions of CAR provided a detailed review and discussion of a large body of data on CAR activation in rodents. The Workshop report concluded with the following statement:

"In conclusion, from an evaluation of literature data a robust MOA based on CAR activation for PB[phenobarbital]-induced rodent liver tumor formation has been developed. The data on species differences was considered by the majority of the panel to be sufficient to determine that this MOA would be qualitatively not plausible for humans. Thus compounds that cause rat or mouse liver tumors through this CAR-mediated MOA, similar to PB, would not be expected to increase the risk of liver tumor development in humans" (Elcombe *et al.*, 2014).

Finally, Ayotte *et al.* (2005) did an extensive analysis of a fish-eating population living on the Lower North Shore region of the St. Lawrence River with an unusually high body burden of polychlorinated biphenyls (PCBs) and dioxin-like compounds (DLCs). They measured biomarkers indicative of liver enzyme induction via CAR, PXR and AhR, and investigated the relationship with organochlorine body burden in adult volunteers. The results of their analyses led to the following conclusion: *"In summary, we found no relation between biomarkers of OC [organochlorine compound] exposure and markers of hepatic enzyme induction in this highly exposed group of fish eaters from the Lower North Shore of the St. Lawrence River."* Given the very high levels of PCB exposure in this high fish-eating population, relative to Spokane River sport fish consumers (see Section II.A), the lack of any notable change in biomarkers of drug metabolizing enzymes provides further proof that activation of CAR, PXR and/or AhR does not occur at the doses of PCBs provided through consumption of fish, even those containing relatively high levels of PCBs.

- **Conclusions regarding Mode of Action for PCB carcinogenesis**

In conclusion, there is strong scientific consensus among the leading toxicologists in the world, regarding the mode of action of PCBs in causing cancer in experimental animals. The key conclusions about the carcinogenicity of PCBs from these reviews are:

1. Mutagenesis/genotoxicity is not a significant contributor to the carcinogenicity of PCBs in experimental animals, and does not contribute to any theoretical cancer risk in humans from exposure to PCBs via consumption of fish;
2. The mixture of PCB congeners found in fish are tumor promoters; they are non-genotoxic and thus initiation is not a significant component of the carcinogenic response to PCB mixtures seen in rats;
3. Although activation of the rat CAR and/or PXR receptors by non-DL-PCBs is likely to contribute to tumor promotion in rat liver seen in some Aroclor studies (e.g., 1016 and 1260), this mode of action is not relevant to liver tumor development in humans, particularly at levels of exposure that are well below the level needed for significant CAR and/or PXR activation.
4. The mode of action for tumor promotion by PCBs is based largely on the presence of dioxin-like PCBs and activation of the AhR is the key molecular event in tumor development in rats. This mode of action is sufficient to explain liver tumors in rats and mice following exposures to mixtures of PCBs that contain DL-PCBs that activate the AhR in rodent liver. While this mode of action is potentially applicable to humans, as I explain elsewhere, the doses to which humans are exposed are too low

to activate the AhR, which is an essential, but not sufficient, step in the ultimate development of PCB-induced tumors, including cancer.

5. For the mixtures of PCBs found in fish, activation of AhR will occur at exposures far below the exposures needed to activate CAR and/or PXR, and thus utilization of AhR activation as the 'Point of Departure' for MOE estimations fully accounts for tumor promotion that might occur at higher doses via CAR and/or PXR activation (e.g., tumors seen in Aroclor 1260-treated rats).

Given the large body of data on mode of action for PCBs and cancer outcomes, the current approach for assessing the potential human cancer risk for PCBs should use a mode-of-action (e.g., Adverse Outcome Pathways) approach. This approach involves modeling the shape of the dose-response curve for the most sensitive Key Molecular Event, activation of AhR in rodents. Since activation of AhR is necessary, but not sufficient, to cause liver cancer in rats, identifying the highest dose that does NOT significantly activate the AhR can then be used as a suitable 'point of departure', or 'threshold' for risk assessment. But because of the well-characterized species differences between human and rat AhR in response to DL-PCBs, it is necessary to use a human TEQ for AhR activation, and then compare the human 'Point of Departure' dose to measured human exposures to DL-PCBs (PCB126) to identify Margins of Exposure for particular exposure scenarios.

4. Dose-response assessment

Dose-response analysis of all of the major 2-year bioassay studies- the NTP studies on PCB126, PCB118 (two tumor types) and TCDD, and the Mayes *et al.* (1998) 2-year studies on Aroclor 1254 and 1260 are plotted in Figure 11.

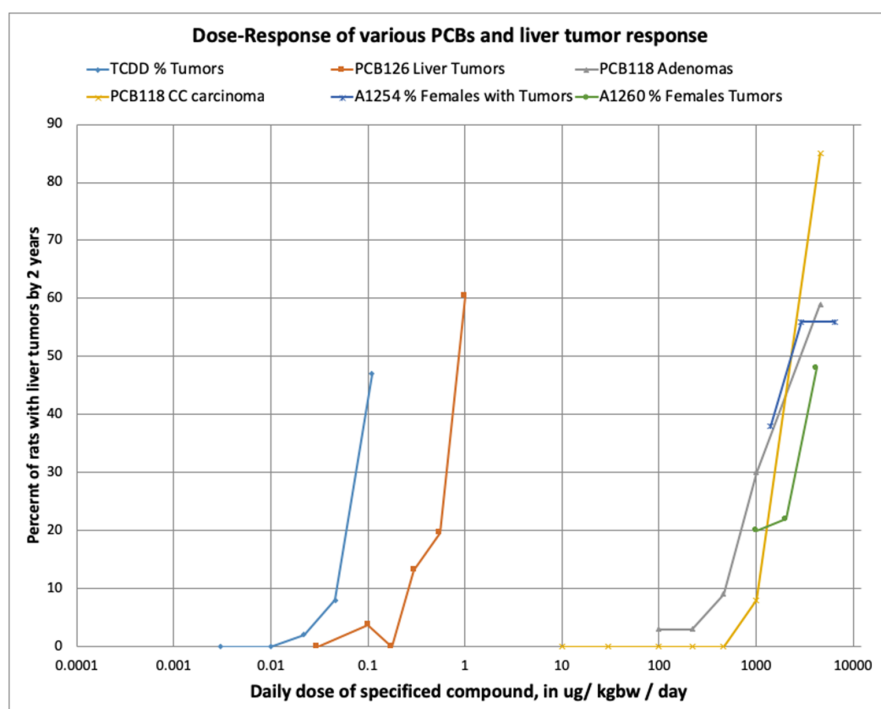


Figure 11. Dose-response analysis of orally administered Aroclors and dioxin-like compounds. Dose is expressed in units of $\mu\text{g}/\text{kg bw}/\text{day}$, and response is plotted as % of animals with benign and/or tumors in the liver. (PCB118 CC = ‘cholangiocellular carcinoma’, a specific type of liver cancer)

Several key points are evident:

1. The slope of the Dose-Response relationship for all compounds is very steep, with tumor response going from 10% to maximal (~50-80% response) occurring over a dose range less than 10-fold, and in some instances less than 5-fold.
2. The steep slope of the dose-response relationship demonstrates the existence of thresholds.
3. The ‘relative potency’ for *in vivo* tumor formation is remarkably consistent with *in vitro* measurements of the various affinities for the AhR, when comparing PCB126 to TCDD (PCB126 is 10-times less potent, and the *in vitro* TEF for AhR binding in the rat is 0.1), PCB118 and TCDD (PCB118 is about 30,000 – 100,000 times less potent than TCDD, and the assigned TEF for PCB118 is 0.00003 (WHO) or 0.000009 (Larsson *et al.*, 2015).
4. Aroclor 1254 used in the Mayes *et al.* (1998) study has a tumor dose-response rate that is similar to what is seen with PCB118 by itself, and the tumor dose-response rate for Aroclor 1260 is less potent than that seen for Aroclor 1254 and PCB118.

Taken together, these ‘daily intake’ dose-response relationships demonstrate the remarkably strong relationship between AhR potency of the various compounds/mixtures, and tumor production over 5 orders of magnitude dose range (from daily doses of TCDD in the 0.01-0.1 $\mu\text{g}/\text{kg}/\text{day}$ range, to Aroclor 1260 doses of 1,000-4,000 $\mu\text{g}/\text{kg}/\text{d}$). If one plots the daily dose in units of TEQ (using *in vitro* estimates based on AhR

induction), the range of responses drops from 5 orders of magnitude (500,000-fold) to about 1 order of magnitude (10-fold) (Figure 12).

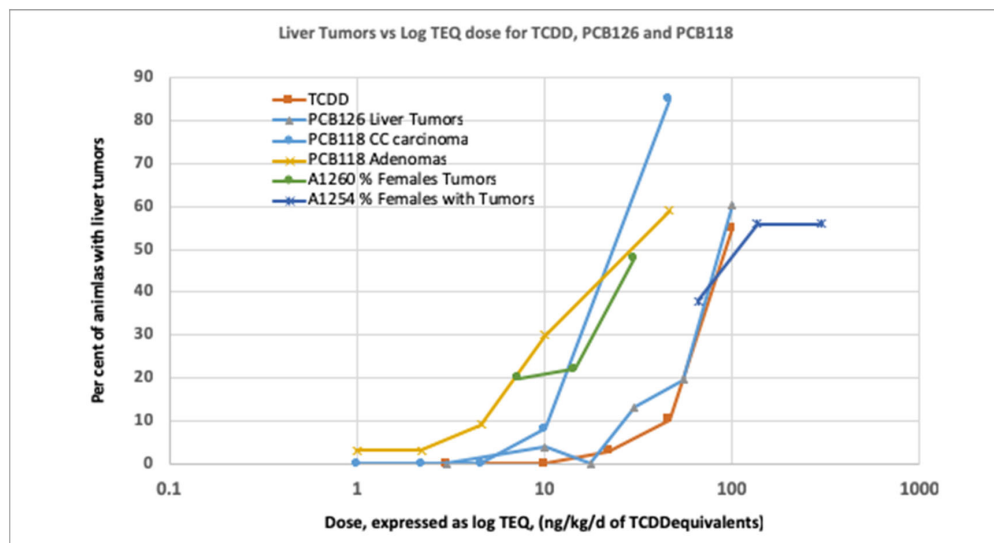


Figure 12. Dose-response relationship between liver tumors and dose of DL-PCBs and Aroclors, normalized to TEQs

These data demonstrate that, in the rat, a lifetime daily dose of TCDD equivalents of 1 ng/kg/d would represent a reasonably conservative 'point of departure' based on tumor responses across a wide range of 2-year bioassays, since in no instance were there tumors noted in any study at doses below 5 ng/kg/d dioxin TEQ (Figure 12). Indeed, Simon *et al.* (2009) conducted a detailed assessment of TCDD tumor response and adverse outcomes pathways and concluded that 1 ng/kg/d of TCDD equivalents was appropriate for use as a Point of Departure based on numerous key events in the development of liver effects following TCDD administration (Table 7).

Table 7. Modeling of various hepatotoxicity end points from TCDD 2 yr bioassays (from: Simon *et al.*, 2009)

Derivation step	Nonlinear Estimates of Cancer Potency to Obtain RfDs based on Combined Liver Tumors and Four Key Events					
	Combined liver tumors		Toxic hepatopathy (15% POD)	EROD (6 × BMD)	AHF (3 × BMD)	Labeling index (0.5 × BMD)
	1% POD (dichotomous Hill model)	1% POD (multistage model)				
Modeled EC _x in rats (tissue concentration, ng/kg)	2610	2475	3085	1566	2493	2497
After application of LN _{AF} (1)	2610	2475	3085	1566	2493	2497
After application of AD _{AF} (0.1)	26,100	24,750	30,850	15,660	24,930	24,970
Modeled HED from Carrier model (external dose, ng/kg/day)	1.3	1.1	1.5	0.8	1.2	1.2
RfD (ng/kg/day) after application of HK _{AF} (3) and HD _{AF} (3)	0.1	0.1	0.15	0.08	0.1	0.1
RfD (mg/kg/day) (commonly used units)	1 × 10 ⁻⁷	1 × 10 ⁻⁷	1 × 10 ⁻⁷	8 × 10 ⁻⁸	1 × 10 ⁻⁷	1 × 10 ⁻⁷

However, they did not have available to them the detailed AhR species response data from Larsson *et al.* (2015) for their species adjustments to arrive at a human-relevant RfD.

Thus, one next needs to consider other factors that might modify this 'Point of Departure' estimate and use an Adverse Outcome Pathway approach (described in Appendix 3C) to establish a Benchmark Dose based on the key molecular event, activation of the human AhR.

5. Risk characterization: Cancer risk from exposure to PCBs found in fish in the Spokane River system

Sub-opinion: Recent studies have demonstrated that only one PCB congener, PCB126, has any measurable activity towards the human AhR, although there are 12 PCBs (so-called 'dioxin-like PCBs') that have appreciable action toward the rat AhR and thus contribute to rat liver tumors in the studies used in the EPA risk assessment. Thus consideration of the daily intake of PCB126 in Spokane River fish is all that is needed to assess MOEs.

As summarized above (and discussed in detail in Appendix 3), since the mode (mechanism) of action of rat liver tumor formation from PCBs requires activation of the AhR, it is important to consider how well the 12 dioxin-like PCBs interact with the HUMAN AhR, since it is human cancer risk that is of interest. The EPA used liver tumors identified in rats fed relatively large doses of PCBs to predict human cancer risk. The default assumption used by the EPA is that humans are at least as sensitive, if not more, than rats, to the cancer-causing effects of PCBs. But the EPA also encourages consideration of differences in potency and adjusting appropriately for those differences when 'mode of action' data are available (US EPA, 2005).

The study by Larsson *et al.* (2015) perhaps provides the most important new information to come from over 14,000 + research articles on PCBs since the EPA's 1996 guidance document. It has been known for more than two decades that human tissues seem to be much less responsive to some 'dioxin-like' molecules when compared to rats (See Appendix 3). However, a careful, quantitative determination of exactly how different human tissues, particularly liver (the site of the tumors that EPA uses in their risk assessment to regulate PCBs in the environment) respond to the various dioxin-like PCBs had not been done until this study.

Larsson *et al.* (2015) used human liver cells and compared them directly with rat liver cells for their responsiveness to AhR induction via various different dioxins and dioxin-like PCBs. They measured EROD activity (discussed above, as the most widely used measure of AhR activation), but also measured other AhR induction metrics, such as the level of mRNA of AhR-driven genes. All of the different measures gave similar results, but EROD was the most 'robust', and is also regarded as the most biologically relevant AhR response. The results reveal three major differences between human and rat AhR response to the DL-PCBs that dramatically change human risk assessments for PCBs from those done by EPA in 1996, and WDOH/ATSDR in 2011, that relied solely on rat (see Table 8 below, reproduced from Larsson *et al.* (2015)): 1) the human AhR is about 25-30 times less sensitive to AhR activation by TCDD, compared to the rat AhR; in other words, it takes at least 25 times more dioxin to cause the same level of activation of human AhR as the rat AhR; 2) only one DL-PCB, PCB126, was capable of activating the human AhR, even though the other DL-PCBs activated the rat AhR with relative potencies very similar to previous WHO determinations; and 3) while PCB126 was able to activate human AhR, it did so with a relative potency in human liver cells approximately 300 times less than TCDD activation in human liver cells, giving a human TEF value of 0.003, rather than 0.1 for the rat AhR.

Larsson *et al.* (2015) then utilized these data to reevaluate appropriate 'in species' TEF values and recommended new TEFs be used for Risk Assessment of dioxin-like compounds, including DL-PCBs.

Table 8. Comparison of the relative potency of various dioxins and dioxin-like PCBs to activate the AhR from different species, including rats and humans (from Larsson *et al.*, 2015)

compound ^b	rat liver epithelial cells		rat lung epithelial cells		rat liver		rat liver	mouse liver	guinea pig liver	human liver		human keratinocytes	
	Cyp1a1 mRNA ^c	Cyp1b1 mRNA ^c	Cyp1a1 mRNA ^c	Cyp1b1 mRNA ^c	EROD primary hepatocytes ^e	EROD H4IIE hepatoma cells ^e	Luc. ^h	Luc. ^h	Luc. ^h	EROD Primary hepatocytes ^e	Luc. HepG2-AZ-AhR cells	CYP1A1 mRNA	AhR mRNA
Chlorinated Dibenzo- <i>p</i> -dioxins													
2378-TCDD	0.020	0.0019	0.0062	0.0032	0.0042	0.0038	0.0056	0.011	0.0015	0.11	0.19	0.12	0.10
Non-ortho PCBs													
PCB77	2.4	0.13	1.1	0.28	9.28	67	40	6.0	0.71	— ^c	— ^c	— ^c	— ^c
PCB126	0.18	0.0030	0.027	0.0081	0.073	0.041	0.076	0.24	0.0081	26	58	50	— ^c
PCB169	2.6	0.058	1.38	0.50	5.8	4.7	2.6	21	0.11	— ^c	— ^c	— ^c	— ^c
Mono-ortho PCBs													
PCB74	14	0.17	1.4	0.26	— ^c	— ^c	4.2	4.5	1.1	— ^c	— ^c	— ^c	— ^c
PCB105	1.8	0.045	0.62	0.089	0.80	— ^c	1.3	6.1	0.012	— ^c	— ^c	— ^d	— ^d
PCB118	— ^c	— ^c	0.56	0.15	1.3	— ^c	1.6	2.9	0.13	— ^c	— ^c	— ^d	— ^d
PCB156	0.20	— ^c	0.046	0.0071	0.039	0.081	0.048	0.28	0.011	— ^c	— ^c	— ^d	— ^d
PCB167	— ^c	0.46	5.0	0.45	— ^c	— ^c	1.5	7.8	0.12	— ^c	— ^c	— ^d	— ^d
PCB189	5.5	0.072	0.87	0.085	— ^c	— ^c	— ^c	— ^c	0.12	— ^c	— ^c	— ^d	— ^d
Di-ortho PCB													
PCB153	— ^c	— ^c	— ^c	12	— ^c	— ^c	— ^c	— ^c	— ^c	— ^c	— ^c	— ^d	— ^d

^aData for primary human lymphocytes and primary murine splenic cells are provided in the Supporting Information (Table S3). ^bNames of compounds are abbreviated as described in section 2.1. ^cThe induced response was too weak to calculate a BMR_{20TCDD} value (see section 2.3). ^dCompound was tested at a concentration of 5 μ M after 3, 6, 12, and 24 h but achieved no induction and was therefore not tested further. ^eBrenerová *et al.* (unpublished results). ^fKrčková *et al.* (unpublished results). ^gLohr *et al.* (unpublished results). ^hGhorbanzadeh *et al.* (2014). ⁱThe coefficient of determination (R^2) was too low (see section 2.3).

Table 9 compares the previous WHO TEFs with the new values recommended for use, based on the results of their study. Of particular interest is that **PCB126 is the only DL-PCB with any measurable activity toward the human AhR**, and thus is the only DL-PCB that need be considered for human-based risk assessments for PCBs. It is important to note that the TEF_{human} values allow for comparison of TCDD-equivalence activities toward the human AhR, but they do not correct for the large species difference in response to TCDD between rats and humans. **Thus, based on the data shown in Table 8 from Larsson *et al.* (2015), an additional 'species correction factor' of ~26 (calculated as the ratio of the human TCDD BMD20 response of 0.11 nM to the rat BMD20 of 0.0042 nM) should be used when using rat data to predict human response.** While I am relying on the Larsson *et al.* (2015) paper for specific cross-species correction factors, it should be recognized that there are multiple papers over the past 15 years that confirm the remarkable difference in AhR responsiveness to various dioxin-like compounds between humans and rodents. These papers, and a thorough evaluation of the Larsson *et al.* (2015) paper, can be found in Appendix 3D.

Table 9. New Consensus Toxicity Factors for Compounds with World Health Organization Toxic Equivalency Factors (WHO-TEF values represent 2005 designation) (from Larsson *et al.*, 2015)

compound ^a	CTF		WHO-TEF
	rat	human	
Chlorinated Dibenzo- <i>p</i> -dioxins			
2378-TCDD ^b	1	1	1
12378-PeCDD ^b	0.5	1	1
123478-HxCDD ^c	0.2	0.03	0.1
123678-HxCDD ^b	0.06	0.06	0.1
123789-HxCDD ^c	0.3	0.002	0.1
1234678-HpCDD ^b	0.04	0.2	0.01
OCDD ^c	— ^c	0.005	0.0003
Chlorinated Dibenzofurans			
2378-TCDF ^b	0.2	0.1	0.1
12378-PeCDF ^c	0.2	0.6 ^{ex}	0.03
23478-PeCDF ^b	0.2	1	0.3
123478-HxCDF ^b	0.09	1	0.1
234678-HxCDF ^b	0.07	0.06	0.1
123678-HxCDF ^c	0.07	0.04 ^{ex}	0.1
123789-HxCDF ^c	0.3	0.02	0.1
1234678-HpCDF ^b	0.01	0.01	0.01
1234789-HpCDF ^b	0.05	0.3	0.01
OCDF ^c	0.007 ^{ex}	0.2 ^{ex}	0.0003
Non- <i>ortho</i> -substituted PCBs			
PCB77 ^b	0.0004	— ^d	0.0001
PCB81 ^c	0.0002	— ^d	0.0003
PCB126 ^b	0.09	0.003	0.1
PCB169 ^b	0.002	— ^d	0.03
Mono- <i>ortho</i> -substituted PCBs			
PCB74 ^b	0.000004	— ^d	—
PCB105 ^b	0.00001	— ^d	0.00003
PCB114 ^c	0.00006	— ^d	0.00003
PCB118 ^b	0.000009	— ^d	0.00003
PCB123 ^c	0.000009	— ^d	0.00003
PCB156 ^b	0.00008	— ^d	0.00003
PCB157 ^c	0.00003	— ^d	0.00003
PCB167 ^b	0.000007	— ^d	0.00003
PCB189 ^b	0.000007	— ^d	0.00003

Footnotes for Table 9:

a) Names of compounds are abbreviated as listed in the Materials and Methods.

b) Based on condensed information (PCA) from the experimental *in vitro* REPs.

c) Based on predictions from QSAR models.

d) No value reported due to the inactivity of PCBs in the human bioassays.

e) No value reported because the compound's membership probability value in the model was too low (below 99% confidence).

Predictions were made at the 99% confidence level (marked "ex") and at 95% confidence level (unmarked). The membership probability values are located in the Supporting Information (Table S7; Larsson *et al.*, 2015).

6. Margins of exposure analysis: MOE for Cancer from PCBs found in edible fish in the Spokane River

Sub-opinion: The doses of DL-PCB TEQ to consumers of fish from the the Spokane River are hundreds of thousands of times below the 'threshold dose' necessary to cause significant (e.g., 20% of maximal) activation of AhR, and thus cannot contribute to increased cancer risk in humans.

Since PCB126 is the only congener of the 209 PCB molecules with potential human health implications via the AhR activation pathway (Larsson *et al.*, 2015), it is important to assess exposures to this specific congener via the diet, which requires an estimate of the concentration of PCB126 in dietary sources, such as fish. However, PCB126 is seldom measured in surveys of PCB levels in fish. Further, congeners differ in their rates

of volatility, rates of decomposition by the environment and organisms, and in their propensity to bioaccumulate in the environment and edible tissues of fish. The focus of regulations for PCBs in environmental media (sediment and water) is on those congeners that bioaccumulate in the food chain, and thus can be consumed in the diet, particularly from fish. Many of the lower chlorinated PCBs (mono, bi and tri) are relatively volatile and do not generally bioaccumulate to significant levels in the aquatic environment. Thus, for risk assessments of PCBs in fish, it is necessary to narrow the consideration of congeners to those typically found in edible tissues of fish. Fortunately, there is a great deal of information available that allows one to do this with some confidence, even if the only measurements taken are of 'total PCBs.' **However, as discussed elsewhere (see Section II.A and Appendix 2: Exposure assessment) for the Spokane River, specific measurements of PCB126 concentration in edible fish tissues have been made and are used in this assessment.**

As described in Table 4 (Exposure Assessment), daily intake of both Total PCBs and of PCB126 have been determined based on measured concentrations of PCBs in multiple different species of fish, and consumption rates for these fish have also been determined from surveys of both sport and subsistence fishermen that consume fish from the Spokane River. As discussed previously, the standard approach for quantitative risk assessment for carcinogens that act via non-genotoxic modes of action, which has clearly been established for PCBs through the NTP bioassays discussed above, is to estimate a 'Benchmark Dose (BMD)' or 'Point of Departure (POD)' dose from the animal studies in which there was no adverse biological response. **As discussed in Appendix 3, the daily TCDD TEQ(rat) BMD01 was determined to be approximately 1 ng/kg/day dioxin equivalents (rat), or 1,000 pg/kg/day. This now represents the animal dose equivalent to the Benchmark Dose, or 'Point of Departure' (POD), from which we compare human exposures to obtain Margins of Exposure (MOE) (Table 10).** However, two adjustments to these MOE estimates are necessary, as they are based on the rat BMD. Thus, to convert the BMD in rats to an equivalent human daily dose it is necessary to use a scaling factor to correct for body size difference between rats and humans. It is widely recognized in pharmacology and toxicology that comparing dose rates between species is best done using body surface area (e.g., mg/m² of surface area) rather than body weight (e.g., mg/kg body weight), which is the conventional dose units used in animal studies. This adjustment value (or scaling factor) is approximately equivalent to using (body weight in kg)^{3/4}, which is the widely accepted value used by the EPA (US EPA, 2005). This would decrease the rat daily intake (i.e., increase its potency) by a factor of 4.2 (Nair and Jacob, 2016; US EPA, 2005)¹¹. Thus, one simply divides the rat dose by 4.2 to convert it to human equivalent intake rate 'per kg bw' so that the BMD/POD of 1000 pg/kg/day in the rat becomes 238 pg/kg/day in human dose equivalents. The second adjustment that is necessary is to adjust for the species difference between rats and humans of TCDD activation of the AhR, which, as discussed above, is a value of approximately 26 (Larsson *et al.*, 2015), since the human AhR is ~26-fold less sensitive to activation by dioxin-like compounds. Thus, the rat BMD/POD adjusted for body weight scaling would then be multiplied by 26 to reflect the lower sensitivity

¹¹ Nair and Jacob provide the following conversion formula as a simple way to convert body weight to body surface area for cross species extrapolation: HED (mg/kg)= Animal NOAEL (mg / kg)(1-0.75), where HED is 'human equivalent dose', and the correction factor in the exponent is assumed to be BW(3/4). However, EPA CAG uses BW(3/4) : *"The 3/4 power is consistent with current science, including empirical data that allow comparison of potencies in humans and animals, and it is also supported by analysis of the allometric variation of key physiological parameters across mammalian species"*, (US EPA, 2005, p3.6).

of the human AhR to activation by PCB126 (Larsson *et al.*, 2015) – i.e., it takes 26 times more TCDD to get the same response from the human receptor as the rat receptor. After correction for both the species scaling factor and the difference in species sensitivity to AhR by dioxin (TCDD, or TCDD equivalents, in the case of PCB126), the MOE for the ‘average’ exposure would be approximately 3,600,000. An extreme/impossible daily dose MOE for a hypothetical consumer eating fish at the 95th percentile of consumption of all fish from the Spokane River, and all containing the upper 95th percentile amounts of PCBs measured in edible tissues in the the Spokane River (Table 4) is 400,000 (Table 10).

Table 10. Estimation of Margins of Exposure for different levels of consumption, and different levels of PCB concentration, of fish from the Spokane River

Exposure scenario	PCB126 daily intake, pg/kg/d (80 kg adult)	Estimated dioxin TEQ, using human-derived TEF (hTEF=0.003)	Species differences correction factor (Rat:Human BW SF = 4.2 (rat:human TCDD sensitivity = 26)	Margin of Exposure Using BMD01 for AhR induction as rat POD (1,000 pg/kg/d)
Mean Consump. Mean Conc.	0.57	0.0017 pg/kg/d	0.00028 pg/kg/d	3,600,000
U95% Consump. Mean Conc.	2.7	0.0081 pg/kg/d	0.0013 pg/kg/d	760,000
Mean Consump. U95% Conc.	1.0	0.0031 pg/kg/d	0.00050 pg/kg/d	2,000,000
U95% Consump. U95% Conc. ¹²	5.1	0.015 pg/k/d	0.0025 pg/kg/d	400,000

Figure 13 illustrates the large difference in exposures between rats receiving PCB126 in their diets in the NTP study, and humans receiving PCB126 in their diet via consumption of fish from the Spokane River. The steep ‘dose-response’ for both AhR induction (EROD Activity, blue line) and rat liver tumor development (orange line) is illustrated on this figure.

When adjusted for species differences, it is evident that even the upper bound ‘worst case’ human exposures are hundreds of thousands of times below the dose that produces any measurable effect on the AhR. Thus, even when considering interindividual variability in response to AhR induction by PCB126 (likely to be less than 10-fold), it is essentially impossible that the dioxin-like PCBs in Spokane River fish could have any biological effects in humans via activation of the AhR at these levels of exposure to PCBs in Spokane River fish.

¹² The combined 95%-ile for both consumption and 95%-ile for fish tissue concentration is a hypothetical/impossible scenario for a consumer because it corresponds to (1) a lifetime of consuming fish at extremely high intake rates, (2) a lifetime of daily consumption of only fish with the highest PCB levels, and (3) concentrations associated with raw fish tissues (because no correction factor was included to account for loss of PCBs that occurs during the process of cooking fish).

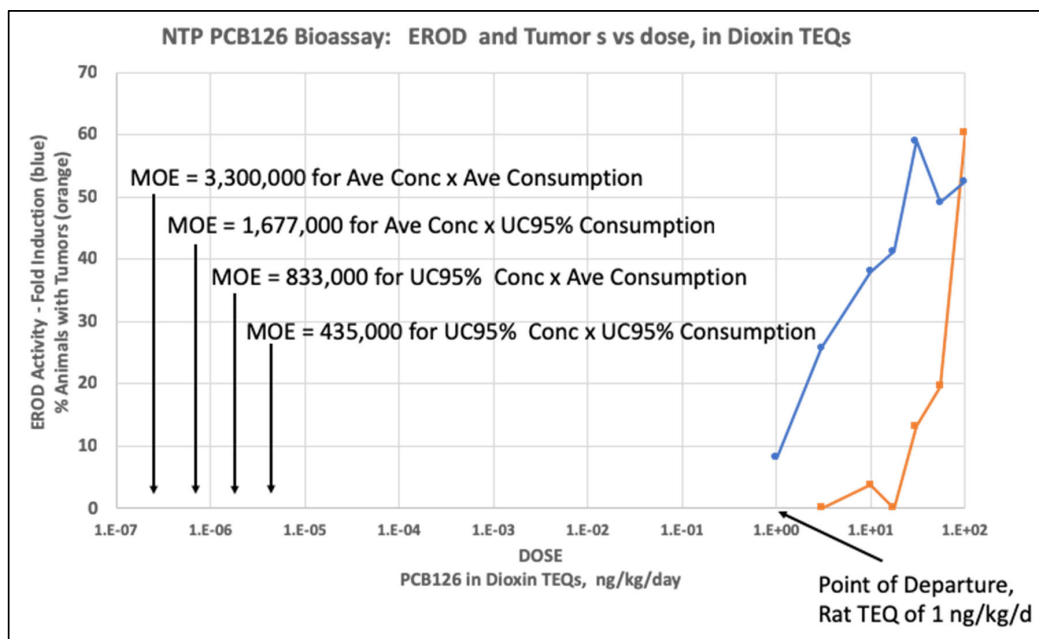


Figure 13. Dose comparison between human TEQ exposure from consumption of fish from the Spokane River to PCB126 dose in NTP Rat bioassay. Blue line represents AhR induction in rats given PCB126 daily in their diets over a lifetime (2 years), and the orange line represents the % of female rats that developed liver tumors after 2 years of PCB126

The results of this Margin of Exposure analysis of PCB126 above indicates that it is essentially impossible that dietary exposures to PCBs could result in significant activation of the AhR in human populations, even in populations with much larger exposures than would occur from Spokane River fish consumption. Although several studies have demonstrated AhR induction in humans following accidental exposures to relatively high levels of chlorinated dioxins and dibenzofurans (e.g., Seveso, Italy reactor accident; Yusho Disease), these are irrelevant to dietary exposures to PCBs via fish consumption because the exposures included large amounts of dioxins and dibenzofurans, rather than just DL-PCBs (Lucier *et al.*, 1987). Using the new consensus TEF values (Larsson *et al.*, 2015), the contribution of PCBs (i.e., PCB126) to the total human TEQ would be very small. As demonstrated by Larsson *et al.* (2015) and many others, the human AhR is indeed highly responsive to dioxins and dibenzofurans, but non-responsive to dioxin-like PCBs, except for PCB126 and possibly PCB81 (see Appendix 3 for details).

Human ‘in vivo’ studies that demonstrate that even relatively high dietary exposures to PCBs do not cause measurable activation of human AhR and subsequent changes in gene expression

Brown (1994) used gas chromatographic analysis of tissues to establish a “fingerprint” of PCB-related metabolites that could be used to demonstrate if exposure to PCBs caused a measurable change in drug metabolizing enzymes, including the AhR-mediated CYP1A1, as well as other CYP enzymes now known to be regulated by CAR and PXR (CYP2B and CYP3A). Using this analytical technique, they measured PCBs and their metabolites in the blood of 138 capacitor workers occupationally exposed to Aroclors, who originally had

serum Aroclor levels averaging 1,655 ppb, a level that is over 2,000 times higher than average PCB blood levels seen in the US population (~0.82 ppb; NHANES data, Patterson *et al.*, 2009), and more than 500 times the 95th percentile of the NHANES data (3.5 ppb; Patterson *et al.*, 2009). Even in this highly PCB-exposed population, they “found no individuals with the clear depressions in PCBs 70 and 118 that would indicate CYP4501A1 induction”, providing further evidence of the relative resistance of humans to DL-PCB-mediated activation of the human AhR.

Pereg *et al.* (2002) was the only study identified that directly measured EROD activity (a consequence of AhR activation) in human tissues following dietary exposures to relatively high levels of PCBs in fish. The authors set out to:

“determine if the body burden of PCBs is related to placental CYP1A1 activity in Inuit women from Nunavik (northern Québec), a population highly exposed to organochlorines [PCBs]. Placenta and cord blood samples were obtained from 35 Inuit women and 30 women from a southern Québec community exposed to background levels of organochlorines. [They] measured PCB concentrations in all cord plasma samples and in a subset of placenta samples from the Nunavik group and assessed CYP1A1 activity (ethoxyresorufin-O-deethylase; EROD) in placental microsomes from all participants.”

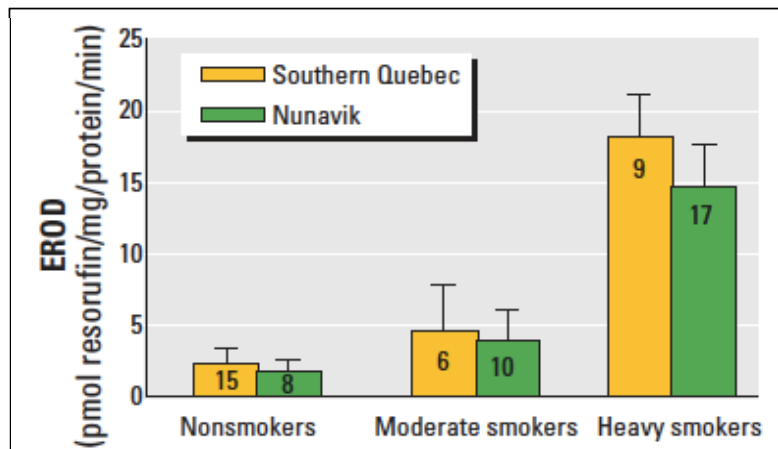
Pereg *et al.* (2002) found that EROD activity in placental tissues of these women is indeed responsive to induction by AhR ligands, since there was a dose-related increase in placental EROD activity when segregated by smoking status. Women who were classified as heavy smokers had about a 6-fold increase in placental EROD, compared to non-smokers. Moderate smokers had approximately 2-fold increase, compared to non-smokers (polyaromatic hydrocarbons, or PAHs, are effective activating ligands for the AhR, and are present in cigarette smoke). However, the relatively highly PCB-exposed Inuit women from Nunavik had NO differences in placental EROD activity when compared to women from a suburban Quebec community with background exposures to PCBs, after controlling for smoking (Figure 14).

The authors provided the following conclusion to their study: “...based on results from the present study, it appears unlikely that PCBs found in the Arctic food chain would be involved in such potential adverse developmental effects through an AhR-signaling pathway and placental CYP1A1 induction.”

It is important to note that ‘Arctic Food Chain Exposure’ of these Inuit women was from consumption of Marine mammals as well as from fish. Marine mammals are known to have much higher levels of PCBs in body fat, compared to fish, and contribute substantially to PCB exposures in circumpolar populations that include marine mammals in their diet (Deutch *et al.*, 2004). Thus, these exposures were likely to be hundreds

to thousands of times higher than would occur from consuming fish caught in the Spokane River, yet there was NO evidence of any induction of AhR in this population.

Figure 14.
Placental EROD activity in Nunavik and southern Québec groups by smoking categories. Bars represent the GM and GSD; values shown inside bars = n.



Long *et al.* (2007) also attempted to correlate DL-PCBs with serum EROD (AhR) activity in relatively highly PCB-exposed populations in Greenland and failed to find a positive correlation between DL-PCBs and serum AhR activity. Indeed, the correlation was actually negative. They hypothesized that the negative correlation was due to PCB congeners inhibiting AhR activation but provided no data for that hypothesis.

Finally, Ayotte *et al.* (2005) did an extensive analysis of a fish-eating population living on the Lower North Shore region of the St. Lawrence River with an unusually high body burden of PCBs and dioxin-like compounds. They measured biomarkers indicative of liver enzyme induction (e.g., AhR and CAR activation) and investigated the relationship with organochlorine body burden in adult volunteers. The results of their analyses led to the following conclusion: *"In summary, we found no relation between biomarkers of OC [organochlorine compound] exposure and markers of hepatic enzyme induction in this highly exposed group of fish eaters from the Lower North Shore of the St. Lawrence River."*

Thus, these data provide additional direct human support for the conclusion that human relevant DL-PCBs (PCB126, PCB81) exposures in even highly exposed populations (via fish and marine mammal consumption) are insufficient to cause a biologically significant activation of the AhR, and thus could not present a significant risk for cancer or other AhR-mediated toxic responses from DL-PCBs. Given the very high levels of PCB exposure in these high fish-eating population, relative to Spokane River fish consumers, the lack of any notable change in biomarkers of drug metabolizing enzymes provides further proof that **activation of human CAR, PXR and/or AhR does not occur at the doses of PCBs provided through consumption of fish from the Spokane River, and therefore consumption of fish from the Spokane River provides no increased risk for cancer, even in consumers of relatively large amounts of Spokane River fish.**

7. Comparison of theoretical cancer risks from exposure to other carcinogens present in City of Spokane drinking water

In addition to managing sewer systems and storm drains that enter the Spokane River, the City of Spokane's Water Department also provides drinking water to more than 220,000 people (City of Spokane -

Environmental Programs, 2019). As noted in the City's release of the 2018 City of Spokane Drinking Water Annual Report, "The City is committed to providing safe, clean drinking water to our customers at an affordable price" (Feist, 2019).

In this 2018 annual report, and in previous ones, the City of Spokane states that the "*City meets or exceeds all drinking water standards.*" They also discuss the fact that there are several known human carcinogens present in the City's drinking water, including radon and arsenic (City of Spokane - Environmental Programs, 2019).

Radon-222

Radon is a radioactive element that occurs naturally in soil and is often found in drinking water in regions of the country where soil levels are relatively high. Spokane is one such region. The City of Spokane's 2016 Annual Drinking water Report states the following, regarding measurements of radon in City drinking water (City of Spokane - Environmental Programs, 2017):

"The Water Department monitored the Central, Hoffman and Nevada wells for radon in 2016, with results of 434 pCi/L, 439 pCi/L, and 445 pCi/L respectively."

and

"The Environmental Protection Agency has published a proposed rule for regulating the concentration of radon-222 in drinking water. The rule proposes a maximum contaminant level goal (MCLG) of zero, a maximum contaminant level (MCL) of 300 pCi/L, and an alternative maximum contaminant level (AMCL) of 4000 pCi/L. The proposed rule would require that community water supply systems (including the City's) generally would have to comply with the MCL of 300 pCi/L, unless there is a multi-media mitigation program (MMM) in place. With a MMM, the AMCL of 4000 pCi/L would apply."

The 2018 Annual Report states the following (City of Spokane - Environmental Programs, 2019):

"The Water Department monitored the Grace, and Well Electric wells for radon in 2018, with results of 380 pCi/L, and 510 pCi/L respectively."

The US EPA estimates that radon exposure is the second leading cause of lung cancer in the United States, second to cigarette smoking (US EPA, 2019c):

"Radon is the number one cause of lung cancer among non-smokers, according to EPA estimates. Overall, radon is the second leading cause of lung cancer. Radon is responsible for about 21,000 lung cancer deaths every year. About 2,900 of these deaths occur among people who have never smoked. On January 13, 2005, Dr. Richard H. Carmona, the U.S. Surgeon General, issued a national health advisory on radon."

Thus, using both US EPA and Washington Department of Health information, it is evident that the City of Spokane's drinking water presents theoretical cancer risks from Radon 222 that exceed any theoretical cancer risks from PCBs, even using the outdated and incorrect assumptions of 'low dose linear' cancer risk from PCBs.

Inorganic Arsenic

Another example of comparative risks that far exceed the theoretical cancer risk from PCBs, estimated by WDOH/ATSDR, and relied upon by plaintiffs experts, is arsenic, which is found in the City of Spokane drinking water:

From 2016 and 2018 Annual Drinking Water Reports (City of Spokane - Environmental Programs, 2017, 2019):

“The arsenic readings in 2016 at the Central, and Well Electric wells were 3.49 and 5.07 ppb respectively. The Maximum Contaminant Level (MCL) for Arsenic is 10 ppb.

The arsenic readings in 2018 at the Nevada, Parkwater and Ray Street wells were 2.77 µg/L, 3.18 µg/L and 3.86 µg/L respectively. The MCL for arsenic is 10 µg/L, or parts per billion (ppb). For City drinking water, 5.13 µg/L of arsenic in 2009 from Ray Street Well represents the highest result to date.”

City of Spokane drinking water currently meets EPA’s revised drinking water standard for arsenic. However, it does contain low levels of arsenic. EPA’s standard balances the current understanding of arsenic’s possible health effects against the cost of removing arsenic from drinking water. EPA continues to research the health effects of low levels of arsenic, which is known to cause cancer in humans at high concentrations and is linked to other health effects such as skin damage and circulatory problems. Information on arsenic in drinking water, testing methods, and steps you can take to minimize exposure is available from the Safe Drinking Water Hotline or at safewater.zendesk.com/hc/en-us/sections/202366558-Arsenic.”

It would be instructive to compare the theoretical risks of consuming Spokane River Fish, as detailed in the WDOH/ATSDR 2011 risk assessment, with the theoretical cancer risk from drinking Spokane City water at the levels described above (~3-5 ppb), using the same approach.

The EPA IRIS document on arsenic provides the following ‘risk’ values for oral exposure to arsenic (US EPA, 1995):

“II.A.1. Weight-of-Evidence Characterization

Classification — A; human carcinogen

***Basis** — based on sufficient evidence from human data. An increased lung cancer mortality was observed in multiple human populations exposed primarily through inhalation. Also, increased mortality from multiple internal organ cancers (liver, kidney, lung, and bladder) and an increased incidence of skin cancer were observed in populations consuming drinking water high in inorganic arsenic.*

II.B. Quantitative Estimate of Carcinogenic Risk from Oral Exposure

II.B.1. Summary of Risk Estimates

Oral Slope Factor — 1.5E+0 per (mg/kg)/day

Drinking Water Unit Risk — 5E-5 per (µg/L)

Extrapolation Method — Time- and dose-related formulation of the multistage model (U.S. EPA, 1988)”

Using EPA's default assumption of consumption 2 liters drinking water per day (US EPA, 2011b) and their 'Unit Risk' (per micrograms/liter) value of 5 additional cancers per 100,000 people exposed at that level, then consumption of City of Spokane drinking water containing 4 ppb ($\mu\text{g/L}$) of inorganic arsenic would result in 8 μg of As per day, and a theoretical increase in 40 cases of cancer per 100,000 people exposed (or 4 in 10,000). **This level of theoretical cancer risk far exceeds the theoretical PCB-related cancer risks from consuming Spokane River fish calculated by Plaintiffs' experts and the WDOH/ATSDR 2011 risk assessment.**

It is important to recognize that hundreds of thousands of people in the City of Spokane do consume 1-2 liters of City of Spokane drinking water on a daily basis, potentially for nearly all of their lives. Further, these EPA risk estimates for both radon and arsenic are based on extensive human studies where exposures were often only 1-2 orders of magnitude greater than City of Spokane residents receive on a daily basis.

In contrast, the 'population at risk' for PCBs is limited only to those people who consume fish caught from the Spokane River on a weekly basis for most of their lives. The size of this 'at risk population' can only be a small fraction of the entire population of Spokane.

If the City's primary motivation for spending hundreds of millions of dollars to reduce the input of PCBs into the Spokane River (so that the concentrations of PCBs in fish would eventually be reduced by 5.5-9%) is public health protection, they would have much greater risk reduction and public health benefit by spending their money on implementing treatment process to reduce the presence of radon-222 and arsenic in their drinking water.

C. Assessment of potential toxicity to the immune system from PCBs in fish in the Spokane River (see Appendix 4 for supplementary materials)

The immune system is composed of a series of balanced, complex, multicellular, and physiological mechanisms whose role is to preserve the integrity of the host. Functionally, the immune system distinguishes "self" tissues (organs and cells) from foreign ("non-self") materials (e.g., bacteria, viruses, transformed cells) and then utilizes one or more of its specialized and complex systems to neutralize and/or eliminate the foreign materials. The immune system operates as a continuum, and perturbation to the system by xenobiotics (i.e., foreign chemicals) may lead to altered immune competence. Changes leading to enhanced responsiveness (or failure to recognize self) can progress to autoimmune disease or hypersensitivity, while decreased ability to recognize (or neutralize/eliminate) foreign material can lead to immunosuppression and illness (Kaplan *et al.*, 2013). Substances are considered immunotoxic when they adversely affect normal immune system operations.

Primary lymphoid organs (bone marrow, thymus) produce and support production of immune cells (e.g., B and T cells). Secondary lymphoid organs (spleen and lymph nodes) filter antigens from the blood and body fluids; other secondary lymphoid tissues are associated with the skin, mucosal lamina propria, gut, bronchioles, and nasal cavity (Kaplan *et al.*, 2013). Functionally the mammalian immune system is comprised of two divisions: (1) innate immunity, which is nonspecific in nature, involves responses by neutrophils, macrophages, natural killer (NK) cells, and dendritic cells (DCs); and (2) acquired (or adaptive) immunity, which is characterized by specificity and immunological memory and subdivided into (a) humoral immunity, which depends on production of antigen-specific antibody B cells and their subsequent interactions with other cells of the immune system and (b) cell-mediated immunity (CMI), which encompasses acquired

immunity not dependent on antibody involvement. Many different types of assays have been used to assess immune system status following exposure to different test articles. In general, these assays evaluate various aspects of immune response (e.g., antibody production or resistance to infection) of control animals compared to responses in groups of animals previously treated with different concentrations of test article. Types of assays used to assess immune function include assays that evaluate general gross immunopathology changes (organ weight and gross cellular changes), humoral (factors in the blood) immunity, cell-mediated immunity, non-specific/innate immunity, overall assessments of immune status (e.g., host resistance assays), and general investigations of factors associated with immune function. Additional parameters regarding the immune system, its components, and analyses assays are provided in Appendix 4.

1. Hazard identification

In 1994, the US EPA included PCB-related immune effects in animal studies as a critical effect for derivation of the human reference dose (RfD) for Aroclor 1254.¹³ This RfD for Aroclor 1254 was based on immunosuppression observed in rhesus monkey studies (i.e., decreased IgG and IgM antibody response to injection of sheep red blood cells, or SRBCs, of female rhesus monkeys given Aroclor 1254 contained in gelatin capsules at doses of 0.005 to 0.080 mg/kg-d for over five years) (Arnold *et al.*, 1993a; Arnold *et al.*, 1993b; Tryphonas *et al.*, 1989; Tryphonas *et al.*, 1991a; Tryphonas *et al.*, 1991b; US EPA, 1994).

A comprehensive data search and analysis to assess the scientific literature for a potential immunotoxicological hazard associated with PCB exposure was performed. As described in Appendix 4, a sequential data search was performed to identify all published immunotoxicity studies of PCBs and Aroclors performed in laboratory animals and all pertinent data that could be used to predict human toxicity was summarized. The literature review resulted in identification of 398 individual immunotoxicity tests that represented 87 individual studies (publications). If the study results found a concentration associated with a no observable adverse effect level (NOAEL) for immunotoxicity, captured information included the highest NOAEL observed. The lowest observable adverse effect level (LOAEL) for immunotoxicity was captured for each test when available. Results were sub-categorized by type of immunotoxicity tests, including gross immunopathology (organ weight/histopathological) changes; humoral immunity; cell-mediated immunity; nonspecific (innate) immunity; host resistance; cell subset distribution changes; and a final category to capture other endpoints beyond those listed.

An overall summary and a table of all reviewed immunotoxicity tests are provided in Appendix 4. In general, some laboratory animal studies observed immune-related effects following PCB exposure, although results sometimes conflicted between studies and inconsistent experimental designs (e.g., one or multiple groups vs. controls evaluated, concentration levels, exposure routes, and exposure durations). Issues with inconsistent study designs likely contributed to the large variability and lack of reproducibility between studies. A potential hazard for immune-related effects was supported by the monkey study data summarized above and

¹³ The RfD is the estimate (with uncertainty spanning perhaps an order of magnitude) of the daily oral exposure to the human population (including sensitive subpopulations) that is likely to be without risk of deleterious non-cancer effects during a lifetime US EPA (2011c). *Integrated Risk Information System (IRIS) Glossary. Terminology Services*. Available at: https://iaspub.epa.gov/sor_internet/registry/termreg/searchandretrieve/glossariesandkeywordlists/search.do?details=&vocabName=IRIS%20Glossary. Accessed October 14, 2017.

by observed immune-related effects (gross effects to lymphoid organs) in laboratory animals treated with high concentrations of PCBs.

2. Mode of action for immunotoxicity

The mode of action for immune-related effects of PCBs is the same as that established for liver tumors in female rats (see Cancer assessment and Appendix 3) – through activation of the AhR. The AhR is commonly expressed in the immune system and is expressed in nearly all immune cell types (Kerkvliet, 2009). The role of AhR in immunosuppression has been well established as dioxin (TCDD)'s immunosuppressant effects on both the innate and adaptive immune systems are mediated through the AhR (Birnbaum and Tuomisto, 2000; Kerkvliet, 1995, 2009; Kreitinger *et al.*, 2016; Preston *et al.*, 1981; Sulentic and Kaminski, 2011; Tian *et al.*, 2015; Tryphonas and Feeley, 2001). Supporting information regarding the role of the AhR in immunotoxicity is available in Appendix 4.

The role of the AhR in modulating the immunotoxicity of PCBs was demonstrated through studies using different strains of mice. Due to genetic differences in the AhR gene, the DBA/2 mouse strain expresses AhR with lesser binding affinities for ligands (dioxin or dioxin-like PCBs), resulting in lesser ability to activate downstream events and generate AhR-mediated effects. Thus DBA/2 mice are sometimes referred to as AhR non-responsive mice. In a study evaluating effects of PCB77 (dioxin-like) or PCB52 (non-dioxin-like) congeners in responsive and non-responsive strains of mice, immune-related effects were observed only in AhR-responsive mice treated with the dioxin-like congener PCB77. No effects were observed in either responsive or non-responsive strains treated with the non-dioxin-like PCB52 (Silkworth and Grabstein, 1982). Another study supported the involvement of the AhR in immune-mediated effects as intraperitoneal injections of dioxin-like PCB77 were associated with immune-related effects in mice only when their bone marrow contained the AhR-responsive phenotype (Silkworth *et al.*, 1986).

One source reviewed indicated that non-AhR immunosuppressive effects of PCBs are mediated via PCB metabolism to “arene oxide intermediates capable of alkylating critical cellular macromolecules to form potentially toxic covalently bound substrate-macromolecule adducts” (Tryphonas and Feeley, 2001). However, the referenced publication (Preston *et al.*, 1981) is about PCB-induced hepatic tumors in the rat and does not mention metabolism, immune status, or reference any immunosuppressive effects (Preston *et al.*, 1981). I found no other data to support an adduct-mediated mechanism for PCB immunotoxicity.

Multiple lines of evidence indicate that the immunotoxic effects observed following PCB exposures are also mediated by the AhR: (1) non-responsive AhR strains are not associated with immune-related effects of PCBs; (2) studies have demonstrated PCB exposures generate similar immunosuppressive effects to those observed with TCDD; and (3) non-dioxin-like PCB congeners are not associated with immunosuppressive effects until MOEs exceed 100,000 (See Appendix 4 for details).

3. Dose-response assessment

Dose-response is the fundamental tenet of toxicology (i.e., adverse response increases with dose), so high quality toxicology tests evaluate more than a single exposure concentration (generally three or more with a separate control group) with multiple administrations (for example one time per day over several days, weeks, months, or years). As detailed in Appendix 4, most immunotoxicity studies that evaluated at least three doses with multiple oral administrations failed to find any immunotoxicity response in the treated

animals. Of those remaining, monkey studies observed dose-responses for dose-response trends for both IgM and IgG following sheep red blood cell (SRBC) inoculation in Aroclor 1254 exposed monkeys (Tryphonas *et al.*, 1989; Tryphonas *et al.*, 1991a). In addition, single administrations of high doses of PCBs and related gross changes in immune organs (i.e., thymus and spleen) further support a dose-response relationship between PCB exposure and immune-related effects (i.e., with high doses in laboratory animals).

Importantly, as discussed in the cancer section, the species responsiveness for AhR activation is different between species. The cancer section discussed how the human is less responsive than the rat. As described in the next section, there is also a different AhR sensitivity between monkeys and humans, where the human AhR is less responsive than monkey AhR to DL-PCB activating ligands.

4. Risk characterization

As described above, immunotoxicity following PCB exposure is dependent on AhR activation. In order to assess human risk related to any specific study of laboratory animals, factors were needed to normalize the dose in animals to an equivalent dose in humans. These factors include considerations to account for (1) different abilities of different compounds (congeners or mixtures) to activate the AhR in the tested animal species; (2) differences between AhR responsiveness (activation) in humans compared to the tested animal species; and (3) differences in animal to human body size differences.

As discussed previously in the Cancer assessment (and Appendix 3), differences between AhR responsiveness (activation) in humans compared to the tested animal species has been determined by using side-by-side cultures of animal cells and human cells in specialized assays (Larsson *et al.*, 2015; Silkworth *et al.*, 2005). Different activation propensities between species were derived using the specific assays and results provided in Table 11. These assays demonstrated that **rat, monkey, and mouse AhRs are 26, 10, and 17-times more responsive than the human AhR, respectively.** Further details regarding conversion of NOAEL and LOAEL doses in animals to human equivalent doses (HEDs) are provided in Appendix 4.

Table 11. Correction factors for animal to human dioxin AhR activation differences

Species	Animal to human dioxin AhR activation correction factor	Basis
Rat	26	Larsson <i>et al.</i> (2015): benchmark response for 20% of maximal EROD induction for TCDD in primary hepatocytes. BMR20TCDD ratio: (humans, 0.11 nM) (rats, 0.0042 nM)
Monkey	10	Silkworth <i>et al.</i> (2005): EC50 for EROD activity for TCDD in primary hepatocytes. EC50 ratio: (humans, 0.29 nM) (monkeys, 0.028 nM)
Mouse	17	Larsson <i>et al.</i> (2015): benchmark response for 20% of maximal AhR induction (Luciferase) for TCDD in human HepG2-AZ-AhR cells and mouse H1L6.1c2 cells. BMR20TCDD ratio: (humans, 0.19 nM) (mice, 0.011 nM)

5. Margins of exposure analysis

As previously discussed, a MOE is a simple ratio between a dose at which an effect of interest is observed in an animal study (NOAEL/LOAEL or Benchmark Dose) and the estimated human intake level. MOEs were calculated using HEDs derived from NOAELs and LOAELs in laboratory animal immunotoxicity tests compared to the human exposure estimates derived using Spokane River site-specific data (see Section II.A).

As discussed previously in the Cancer assessment and Appendix 3, the only congener capable of eliciting a human AhR response is PCB126. Because immune effects are due to AhR activation, the only human PCB exposure relevant for potential immune-related effects in humans is PCB126 (See Appendices 3 and 4 for detailed discussions of species differences in AhR activation by DL-PCBs). Human intake of PCB126 from Spokane River fish consumption was estimated using species-specific PCB126 concentrations measured in Spokane River fish tissue together with species-specific consumption data for Spokane River fish (see Exposure Assessment section for details on relevant assumptions). Reasonable upper bound estimates were calculated using (1) the upper 95th population-based percentiles of PCB126 tissue concentrations with the mean levels of Spokane River fish consumption (meaning that 95% of tissue concentrations were at or less than the value used) and (2) mean PCB126 tissue concentrations together with 95th percentiles for Spokane River fish consumption levels (meaning that 95% of consumers' consumption was at or less than the value used). The resulting values were 0.0827 and 0.217 ng/day, respectively (see Section II.A). I used the more

conservative (i.e., higher) value of 0.217 ng PCB126 /day as the predicted human intake of PCB126 in this MOE analysis.

To calculate MOEs specific to immunotoxic endpoints, I used the following equation:

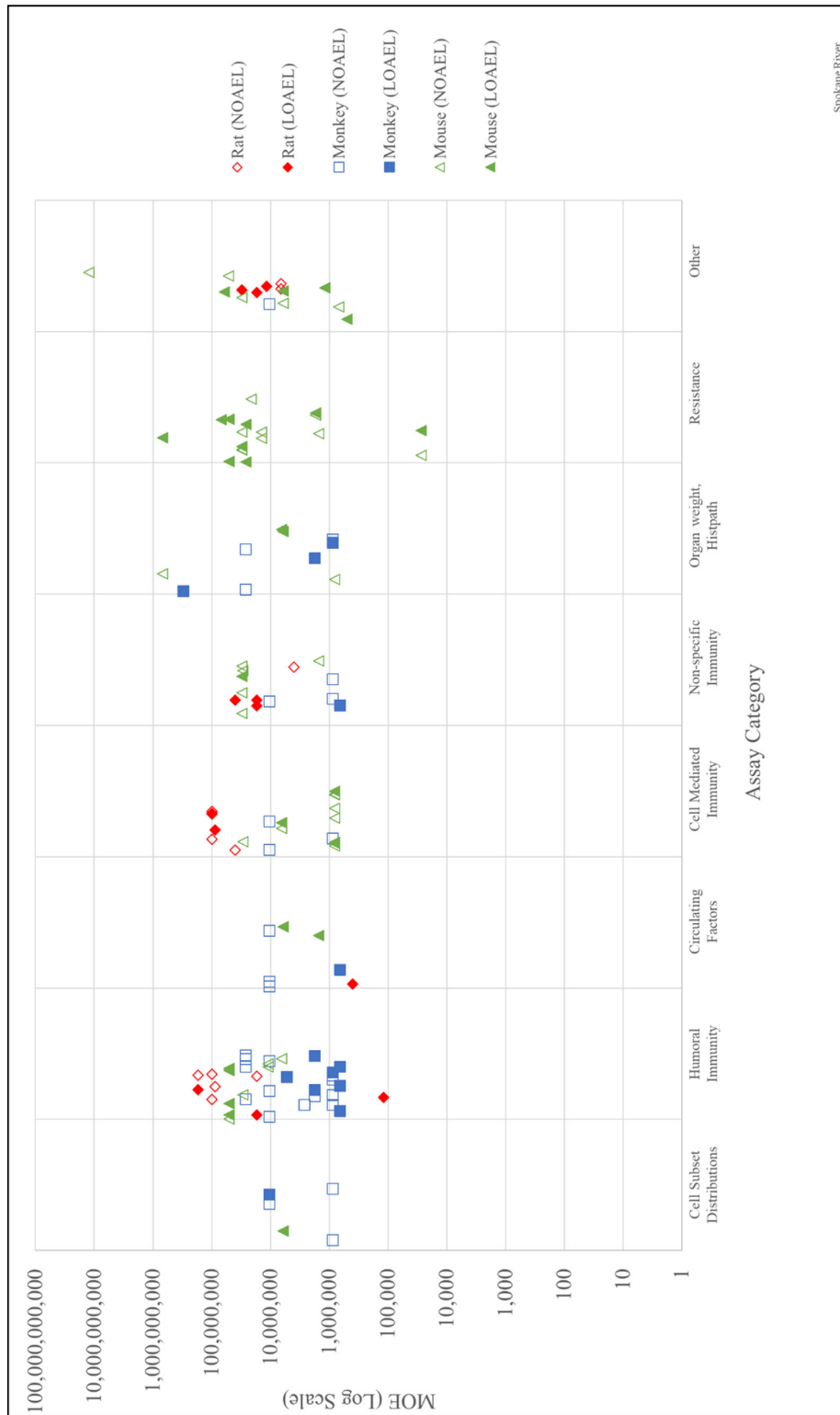
Equation 1. Margin of exposure calculation for dioxin like PCBs

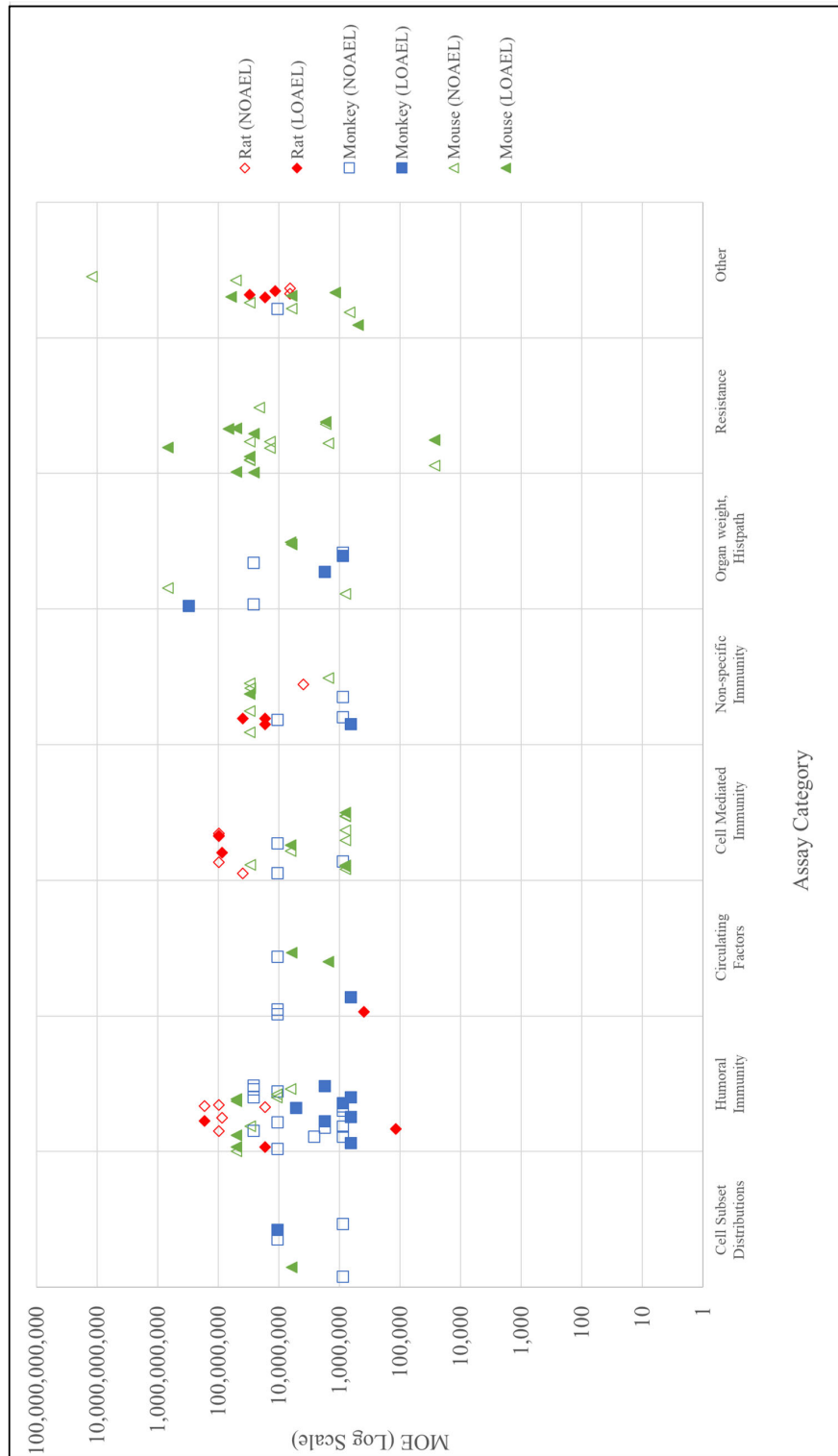
$$MOE = \frac{HED_{Dioxin} \left(\frac{mg}{kg \text{ bw day}} \right)}{[PCB126] \left(\frac{ng}{day} \right) * \left(\frac{1 \text{ mg}}{10^6 ng} \right) * \left(\frac{1}{80 kg} \right)}$$

where HED_{Dioxin} was the human equivalent dose for immune-related NOAELs and LOAELs from laboratory animal studies and [PCB126] was the predicted daily human intake of PCB126, converted to mg/kg-d assuming an average human bodyweight of 80 kg (US EPA, 2011a).

Equation 1 was applied to each of the mouse, rat and monkey studies that used an oral route of administration and two or more dose administrations. Resulting MOEs for each NOAEL and LOAEL are included in Table A4-10 and Table A4-11, respectively. All derived MOEs are presented by immunotoxicity assay category in Figure 15.

Figure 15. Estimated MOEs for Spokane River fish consumption associated with immune-related NOAELs and LOAELs from laboratory animal studies





Regardless if MOEs were derived from NOAELs or LOAELs, the majority of MOEs were greater than 100,000 (and all were greater than 10,000). In order to assess the population of derived MOEs, the mean and lower 95th percentile MOEs were conservatively derived using all NOAEL and LOAEL datapoints for each immune-related assay category and for the entire dataset (i.e., all categories combined; see Table 12). When the entire dataset was considered, the resulting lower 95th percentile MOE was greater than 100,000, which means that the estimated reasonable upper bound estimate of exposure in humans is more than 100,000-times above the levels tested in animal studies and thus do not present a risk of immunotoxicity with ingestion by humans consuming fish from the Spokane River.

Table 12. Mean and lower 95th percentiles for Spokane River MOEs associated with all immune-related NOAELs and LOAELs from laboratory animal studies

Assay category	Mean MOE	Lower 95 th Percentile MOE
Cell Subset Distributions	5,830,000	885,000
Humoral Immunity	31,173,000	664,000
Circulating Factors	5,798,000	478,000
Cell Mediated Immunity	28,923,000	811,000
Non-Specific Immunity	17,460,000	808,000
Organ weight, Histopathology	106,786,000	844,000
Resistance	60,412,000	27,000
Other	835,365,000	631,000
All categories combined	131,187,000	664,000

Resistance assays were associated with the lowest MOEs as both individual data points and as the lowest value for the lower 95th percentiles of assay categories; the lowest two MOEs for resistance assays are two individual data points (one NOAEL, one LOAEL; see Figure 15) that originate from two tests conducted as part of a mouse study (Loose *et al.*, 1978a). The NOAEL corresponded to a host resistance assay to *Plasmodium berghei* infection following an approximate 1 md/kg-d exposure to Aroclor 1016. Exposure lasted for either three weeks or six weeks with no significant difference in mean survival time. As the reported NOAEL was the only dose given, this result simply means that there was no adverse effect observed related to host resistance to *Plasmodium berghei* infection following Aroclor 1016 exposure. The LOAEL associated with the lowest resistance assay MOEs corresponded to a host resistance assay to endotoxin response following an approximate 1 md/kg-d exposure to Aroclor 1016. Exposure lasted for three weeks, and a statistically significant ($P < 0.05$) decrease in LD₅₀ was observed compared to control mice.

Overall, all MOEs related to Spokane River fish consumption and immune-related NOAELs and LOAELs from laboratory animal studies indicated the reasonable upper bound estimate of human exposure was 27,000 times above levels tested in animal studies and thus do not present a risk of immunotoxicity with Spokane River fish consumption.

D. Assessment of the potential risk for adverse neurodevelopment outcomes from PCBs in fish in the Spokane River based on laboratory animal studies (see Appendix 5 for supplementary materials)

1. Introduction to risk assessment for adverse neurodevelopmental outcomes

Based on this assessment of the hazard, exposure, and toxicity of PCBs, and using a weight-of-evidence approach, I conclude that it is unlikely that any adverse neurodevelopmental outcomes will occur from exposure to PCBs in fish caught and consumed from the Spokane River. Results are summarized below and included with more detail in Appendix 5.

I calculated PCB exposure through fish consumption using two different scenarios: a best estimate and a reasonable upper bound. The reasonable upper bound scenario is based on conservative assumptions of the amount of fish consumed from the Spokane River. Using conservative assumptions about exposure is one of many ways a toxicologist can ensure that risks estimated in a human health risk assessment are not underestimated. My reasonable upper bound exposure scenario assumed that every day, a person consumes each species of fish from the Spokane River at a rate that is near the maximum consumption rate for all people who catch and consume this fish (*i.e.*, at the 95th percentile of the consumption rate for each species of fish). This scenario is not plausible; however, this was used as the conservative consumption rate in this assessment for the calculation of the margins of exposure (MOEs) for each study identified as relevant. Thus, the reader should consider the level of conservatism when assessing the results of this toxicological assessment.

The focus of this review is on the toxicological data from experimental animals. Epidemiological studies focused on this outcome have been completed by an expert in Epidemiology (Dr. Michael Goodman).

2. Hazard identification

Numerous investigations since the 1970s have assessed the potential for PCBs, including commercial mixtures (*e.g.*, Aroclors), to cause neurodevelopmental effects in animals, including effects on cognition, behavior, motor skills and sensory systems.

3. Mode of action

This assessment was conducted using a weight of evidence approach of the published studies that have evaluated the relationship between PCB exposures and neurodevelopmental in experimental animals.

Unlike the cancer and immunotoxicity endpoints discussed in this report, the mechanistic basis for the neurodevelopmental effects of PCBs observed in some animal studies is not established. Both DL- and NDL-PCBs have been tested for neurodevelopmental effects in experimental animals. Multiple mechanisms of action for neurodevelopmental effects have been proposed and tested *in vitro*. The MOAs include enhanced ryanodine receptor activity, decreased dopamine content, perturbations in calcium (Ca²⁺) homeostasis, activation of aryl hydrocarbon receptor, protein kinase C (PKC) translocation, and perturbation of G-protein-coupled receptor (GPCR) signaling, among others (Choi *et al.*, 2016; Kodavanti, 2005; Mariussen and Fonnum, 2006; Pessah *et al.*, 2010; Pradeep *et al.*, 2019). For example, PCB95 (a NDL-PCB) has been shown to activate the ryanodine receptor, a Ca²⁺ ion channel expressed by neurons in the mammalian brain, leading to increased intracellular Ca²⁺ levels (Pessah *et al.*, 2010). Changes in intracellular Ca²⁺ is one mechanism by

which neuronal activity regulates neurodevelopmental processes that in turn determine synaptic connectivity (Bal-Price *et al.*, 2017).

4. Dose-response assessment

The overall dose-response assessment, based on a careful analysis of the literature, demonstrates that the PCB doses administered to animals were at levels greater than would be consumed by people eating fish from the Spokane River. A review of the pertinent literature, which includes non-human primates (NHP) and rodent studies, is included below with a brief review of the major limitations of these studies (See Appendix 5 for more detail).

A number of factors limit the ability to interpret dose-response within or across cohorts of NHPs. The factors include that studies were conducted in only two labs and the same cohorts were tested in multiple studies (*e.g.*, without the apparent consideration of the effect multiple tests have on a cohort), individual NHP cohorts rarely included more than one dose group, and few neurodevelopmental tests were administered across multiple cohorts or consistently for a given PCB mixture or at the same ages across multiple studies. In addition, tests defined as cognitive by authors may include measurements that pertain more to behavioral endpoints (*e.g.*, response inhibition) than learning or memory, confounding interpretation of results for certain endpoints.

Overall, interpretation of NHP test results does not support significant effects on learning or memory in either the Health Canada or the University of Wisconsin (UWisc) cohorts of NHPs. A test for response inhibition identified effects in the Health Canada cohort exposed to 7.5 µg/kg-d; these effects are inconsistently supported in comparable measurements on the same cohort or on cohorts dosed with 6.3 or 90 µg/kg-d Aroclor 1248 by UWisc. Effects on motor activity were reported at 90 µg/kg-d Aroclor 1248 but not at lower doses (6.3-13 µg/kg-d) of Aroclor 1248. Effects at the higher dose showed inconsistently high or low levels of activity depending on age.

Studies that administered PCBs to rodents at doses environmentally relevant for the Spokane River (*i.e.*, in the ng/kg-d range) did not identify cognitive or motor effects in rodents. In offspring of mice administered a mixture of six NDL-PCBs in the ng/kg-d range, Elnar (2012) reports significant effects in two tests of anxiety, but these are not replicated in other studies at comparable or significantly higher doses, and they rely on a number of measurements that either do not show dose-response within the study or are not clearly clinically significant. Elnar (2012) also reports a reduction in horizontal (locomotor) activity at 0.001 and 0.010 µg/kg-d, but not at 0.100 µg/kg-d in tests of motor activity in an open field test. Studies that dose at levels greater than 1 mg/kg-d—much higher than environmental exposure levels— report mixed results in tests of learning and spatial memory, as well as overall activity level.

5. Risk characterization and margins of exposure analysis

Because the majority of the 87 animal neurodevelopmental studies that I reviewed (*i.e.*, studies that passed the screening criteria) exposed animals to PCB doses well above environmental exposure levels, the majority of calculated MOEs for both the best estimate and reasonable upper bound scenarios were at or above 1,000 and in most cases were orders of magnitude higher than 1,000. For the best estimate scenario, only two out of the 87 studies conducted neurodevelopmental tests at more environmentally relevant doses (*i.e.*, ng/kg-d to low µg/kg-d range) that identified LOAELs corresponding to MOEs <1,000 (MOEs ranged from 0.181 to

18.1). Both studies were in mice, and LOAELs were for tests assessing behavioral and motor endpoints (e.g., open field activity, elevated plus maze, light-dark box, and sociability). For the reasonable upper bound scenario, seven out of the 87 studies identified at least one LOAEL for a neurodevelopmental test with an MOE <1,000 (MOEs ranging from 0.0410 to 753). Two of these seven studies were the same studies in mice as for the best estimate scenario; the additional five studies were conducted in NHPs, and included LOAELs for tests assessing cognitive/behavioral endpoints (e.g., schedules of reinforcement, discrimination reversal, and delayed spatial alternation).

Overall, results from these studies (including those with MOEs <1,000) showed no or slight differences between treated animals and controls and/or exhibited inconsistent dose-response. Neurodevelopmental PCB studies in animals as a whole lack repeatability, replicability, and reproducibility. This is mainly due to the wide variability in experimental design across studies.

Based on this assessment of the hazard, the conservative assumptions of exposure, and toxicity of PCBs, I conclude that it is unlikely that any adverse neurodevelopmental effects will occur from exposure to PCBs in fish consumed from the Spokane River.

E. Assessment of the potential risk for adverse reproductive outcomes from PCBs in fish in the Spokane River (see Appendix 6 for supplementary materials)

1. Introduction to risk assessment for reproductive outcomes

Based on this assessment of the hazard, exposure, and toxicity of PCBs, using a weight-of-evidence approach, I conclude that it is unlikely that any reproductive or developmental effects will occur from exposure to PCBs in fish consumed from the Spokane River. Results are summarized below with more detail included in Appendix 6. Like the neurodevelopmental assessment, my assessment of the potential for reproductive and developmental effects makes highly conservative, as well as more average, assumptions about the amount of fish consumed from the Spokane River. Thus, estimates of potential reproductive and developmental health risks are likely overestimated and err on the side of caution. The reader should consider the level of conservatism when assessing the results of this assessment.

2. Hazard identification

Numerous investigations since the 1970s have assessed the potential for PCBs, including commercial mixtures (e.g., Aroclors), to cause reproductive and developmental effects in animals. The endpoints reported with sufficient exposure in the animal literature with oral exposure to PCBs include reduced fertility in males and females, abnormal estrous cycling, reduced birthweight in offspring, and increased incidence of still birth (Ahmad *et al.*, 2003; Arnold *et al.*, 1995). Endpoints reported with exposure in males include changes in semen quality parameters (sperm concentration, motility, and morphology), sperm integrity, and circulating reproductive hormone levels (Meeker and Hauser, 2010).

3. Mode of action

Several hypothesized modes of action (MOAs) are reported for reproductive and developmental effects of PCBs. MOAs include activation of the AhR receptor, perturbations of hormones (e.g., thyroid hormone and estrogen), direct interaction with hormonal receptors or degradation of hormones, impairment of signaling pathways, or oxidative stress. Given that no single MOA pathway is evident, I did not use an Adverse

Outcomes Pathway (AOP) approach for my assessment of reproductive and developmental effects and instead relied on weight-of-evidence based on empirical evidence.

4. Dose-response assessment

The overall dose-response assessment, based on a careful analysis of the literature, demonstrates that the PCB doses administered to animals are at levels greater than what would be consumed by people eating fish from the Spokane River. I assessed dose-response data on the effects of PCBs on female and male reproductive endpoints and developmental effects in female and male offspring in NHPs, rats, and mice. The following is my review of the literature, including a brief review of the major limitations of these studies (See Appendix 6 for more detail).

With female reproductive endpoints, variations in response are observed depending on species and exposure (congener or mixture). Overall, the endpoints showing effects at the lowest dose appear to be decreases in conception and live births. A decrease in conceptions is consistently reported in NHPs, mice, and rats, with LOAELs ranging from 0.02-12 mg/kg-d and NOAELs ranging from 0.005-64 mg/kg-d. A decrease in live births is reported in NHPs with LOAELs ranging from 0.02-0.2 mg/kg-d; however, this effect is not reported in rodent studies at a range of doses.

The studies evaluating reproductive effects in males are insufficient for determining a robust point of departure (POD) for evaluating hazard from exposure to PCBs, as there are few studies, a paucity of effects reported, and little consistency across species. Based on the study by Cai *et al.* (2011), which included a range of doses and effects in mice exposed to Aroclor 1254, a POD for male reproductive effects equal to the NOAEL of 0.05 mg Aroclor 1254/kg-d can be identified, based on decreases in sperm quality, testicular weight, and estradiol. The LOAEL for these effects is 0.5 mg/kg-d.

For developmental effects in female offspring, variations in response are observed depending on species, exposure (congener or mixture), and exposure source (gestational or lactational). The endpoints showing effects at the lowest doses for female offspring development are changes in ovarian or uterine weights. However, these effects are inconsistent, with ovarian weights decreased in mice, albeit in only one study, but unchanged in rats, and uterine weights decreased in rats with lactational exposure, but not in either rats or mice with gestational exposure. No effects on pregnancy success were reported in either species. The weight-of-evidence suggests no consistent effect of PCBs on the development of female offspring. Thus, derivation of a POD for developmental effects in female offspring is not justified based on the available data.

For developmental effects on male offspring, variations in response based on species and exposure (congener or mixture) are evident. The endpoint showing effects on male offspring development at the lowest doses appears to be changes in testosterone levels. Decreases in testosterone were reported in both rats and mice, although the only study I identified in mice included only one dose. Rat studies report decreases in testosterone with either gestational or lactational exposure and with different congeners. The lowest doses reported to cause a change in this parameter are 0.03 mg/kg-d PCB169 in rats (Yamamoto *et al.*, 2005) and 0.001 mg/kg-d PCB101+PCB118 in mice (Pocar *et al.*, 2012). Both of these studies included only one dose. For studies with multiple doses tested, the LOAEL was 4 mg/kg-d for a mixture of 14 PCBs (Kaya *et al.*, 2002).

5. Risk characterization and margins of exposure analysis

Two publications were identified that report on the same cohort of animals (NHPs) with reproductive LOAELs that correspond to MOEs between 100-1,000: Arnold *et al.* (1995) and Truelove *et al.* (1990). Arnold *et al.* (1995) reported that conception was decreased in NHPs exposed to 0.02, 0.04, or 0.08 mg Aroclor 1254/kg-d dose groups; the MOE based on a LOAEL of 0.02 mg/kg-d is just below 1,000 (MOE=991). Truelove *et al.* (1990) reported a reduction in progesterone levels in NHP at the lowest dose tested in the study (0.005 mg Aroclor/kg-d). However, while this finding was statistically significant, it was not considered biologically significant by the study authors since the changes did not occur in a dose related manner (the 0.020 and 0.040 mg/kg-d dose groups showed no differences).

Three developmental studies with LOAELs corresponding to MOEs less than 1,000 were identified: Fiandanese *et al.* (2016), Gupta (2000), and Pocar *et al.* (2012). Pocar *et al.* (2012) and Fiandanese *et al.* (2016) are from the same laboratory and use the same exposure mixture (a 50:50 mixture of PCB101 and PCB118) and the same mouse model. No other studies assessed this mixture. For the endpoints reported to be altered at doses corresponding to MOEs less than 1,000, other studies have not reported the same effects at doses orders of magnitude greater. The studies by Gupta (2000) and Fiandanese *et al.* (2016) only tested one dose of Aroclor 1016 or the mixture of PCB101 and PCB118, respectively. Although significant results are presented, studies with only one dose do not allow for an assessment of dose-response under the conditions of the study or a clear assessment of whether the effects are even treatment related. It should be noted that, as stated by the authors, “two PCB congeners used in the present study are either pure CAR inducers (PCB101) or AhR/CAR mixed-type inducers (PCB118).” It has been known for decades that both AhR and CAR-regulated CYPs (e.g., CYP1A and CYP3A) metabolize testosterone. The authors noted that “The dramatic suppression of testosterone induced by the two EDs is likely to underlie most of the reproductive deficiencies observed in the present study, as supported by the fact that reduction of ITT was accompanied by defective sperm production, low semen quality and a significant reduction of the tubules in androgen-dependent stages.” Thus, it is likely that the effects seen in this study were secondary to PCB101/PCB118 induction of AhR and / or CAR. As discussed previously in the Cancer Risk section, this mode of action is largely irrelevant to human risk at dietary levels of exposure to PCBs in fish because PCB118 has no activity toward human AhR, and CAR activation in humans does not occur following exposure to PCBs found in fish, even in high fish-eating populations Ayotte *et al.* (2005).

Thus, these studies do not appear to be reliable for use in assessment of PCB risk at environmental exposure levels.

Based on this assessment of the hazard, the conservative assumptions of exposure, and toxicity of PCBs, I conclude that it is highly unlikely that any reproductive or developmental effects will occur from exposure to PCBs in fish caught and consumed from the Spokane River.

III. Historical context of the toxicology of PCBs and Monsanto's standards of practice

A. Charge 2: Evaluate the state of the science of toxicology for industrial chemicals from the 1930's to the late 1960's, and whether Monsanto's practices were consistent with standard toxicological practices of the time

Opinion: Monsanto's use of toxicological expertise and studies in the 1930's-60s were consistent with industry standards and practices of the time

1. Standards of practice in industrial toxicology, 1930's-1960's

Prior to the passage of the Toxic Substances Control Act (TSCA) in 1976, only chemicals that were intended for use as drugs, food additives or pesticides were required to be tested for carcinogenicity before introduction into commerce, unless there were concerns related to work-place exposures. The Occupational Safety and Health Act (OSHA) encouraged companies to test chemicals in laboratory animals, as concerns were almost exclusively focused on worker safety and short-term (acute) toxicity.

During the mid-1950s, the field of 'industrial toxicology' began to expand, largely due to growing concerns about occupational exposures, and new concerns about human health effects of pesticides and food additives to consumers.

It is informative to evaluate the development of the 'state of knowledge' of industrial toxicology through the eyes of one who 'was there.' In the first edition of the widely-acclaimed toxicology textbook, *Casarett and Doull's Toxicology: The Basic Science of Poisons*, published in 1975, chapter 28, titled 'Industrial Toxicology', was written by Henry F. Smyth, Jr (Smyth, 1975), who was a pioneer in the very small field of industrial toxicology. He worked at the Mellon Institute and was an adjunct professor at the University of Pittsburgh, one of the leading academic institutions in industrial hygiene and safety in the 1930-50's. In this book chapter, he describes the 'state of the science' of industrial toxicology during 1940's, 50's and 60's. Regarding the testing of new chemicals, or other industrial chemicals not intended for use as pesticides, food additives or pharmaceuticals, he states the following:

Simple and expeditious animal test such as are visualized have been described by the author under the name of the range-finding test (Smyth and Carpenter, 1944), and the results of its application to a large number of synthetic organic chemicals have been published (Smyth and Carpenter, 1948; Smyth et al., 1949, 1951, 1954, 1962, 1969a; Carpenter et al., 1974). The details of the test have remained unchanged in all essentials for almost 30 years. Results obtained today on one chemical are quantitatively comparable with those obtained many years ago on another chemical.

Five possible injurious actions of chemicals are explored: the oral LD50 for albino rats; the 24-hr skin penetration LD50 for albino rabbits; the time rats survive inhalation of vapors substantially saturated at room temperature, or if this be less than four hours, the four-hour rat LC50; the primary irritation on the clipped, uncovered albino rabbit belly; and the quantity or concentration required to produce a severe chemical burn on the cornea of albino rabbits. The details of the technique are best described in an article by Smyth and

Carpenter (1944). The five data constitute the range-finding toxicity of a chemical, actually a profile of relative hazards of various exposure to and contacts with the chemical."

It is important to note several points about this excerpt from Dr. Smyth's textbook chapter:

1. This pertains to industrial chemicals not intended for use as pharmaceuticals, food additives, pesticides used on food crops for which other guidelines were developed. PCBs were never marketed and sold for any of these purposes.
2. There is not even a suggestion that two-year bioassays be conducted for the purposes of evaluating potential cancer-causing properties of the materials not used as pharmaceuticals, food additives, or on-crop pesticides.
3. This article was written in the early 1970's (publication date of the book was 1975), so reflects the guidance to the 'next generation' of industrial toxicologists, given the prominence of this book, which is the first toxicology textbook intended for undergraduate and graduate students, as well as practicing professionals of the time.
4. Monsanto conducted all of the types of studies mentioned, and hundreds more, in the 1940's, 50's and 60's (see Attachment 1).

In evaluating the state of science and how cancer testing has evolved since the 1930s, it is important to compare the state of animal cancer testing before and after standardized protocols came to fruition in the 1970s. Certain aspects of study design scientists take for granted today were not regarded as important in the 1930s. Table 13 lists these differences, which include treating the controls in the same fashion as treated animals, understanding the background incidence of disease in the studied experimental animal, and consideration of the age of animals tested, using an adequate number of animals to see a response, testing the animals for long enough periods of time (2 years), as well as numerous issues related to animal care and housing.

Table 13. Typical animal chronic testing study design practices in the twentieth century for industrial chemicals (e.g., non-pharmaceutical and non-food additive/pesticide chemicals)

Study design parameter	Early twentieth century (~1930-1950s)	Later twentieth century (>~1960)
Number of dose groups	Typically a single dose group	Minimum of three or more groups
Method of administration (e.g., dermal, inhalation, gavage, dietary)	Dermal or inhalation exposure (assess occupational exposure)	Dietary or gavage to ensure dose
Length of administration (How Long)	Variable	Established period of time: Lifetime ~ 2 yrs
Animal care	Not standardized	Rigidly controlled, standardized animal medicine practices
Tissue analysis	No uniform classification system	Established classifications
Pathology review	Single pathologist	Multiple pathologists
Statistical practices	None or non-standardized	Highly standardized
Group size	Variable	Larger numbers of animals, animals individually tracked and assessed
Species	Multiple species	Rats or mice, consistent strain or sensitive strain
Gender	Random gender selection	Both genders or most sensitive gender
Age	Varied	Studies begin at specific, young ages
Historical controls (Summary of control animals)	Generally not available	An integral part of study design
Doses administered (total dose and variability within study period)	May have relied on a minimum range finding study (dose could be adjusted during study)	Use of a subchronic study to set chronic dose levels (doses aren't typically adjusted)
Observations	Limited	Comprehensive
Intervals of administration (On/Off)	Variable intervals	Continuous
Test substance	Purity impossible to determine	Purity confirmed, contaminants identified
Source of test compound	Not specified	Well documented
Record keeping	No requirements	Good Laboratory Practice (GLP) regulations
Additional analysis (e.g., hematology, urinalysis)	Limited	Comprehensive analyses
Laboratory design	Not standardized	Clean/dirty corridor systems and Standard Operating Procedures
Study segregation	Not standardized	One study per room

2. The early days of PCB manufacturing: 1930's-1950's: What Monsanto did in response to concerns about workplace safety and reports of serious toxicity from manufacturing processes where PCBs were used

Polychlorinated hydrocarbons, including 'chlorinated naphthalenes' (PCNs) and polychlorinated biphenyls (PCBs) were first used for industrial purposes in the early 1930s because they provided a non-flammable alternative to petroleum hydrocarbon-based liquids.

In 1937, one of the first reports on animal toxicity of these polychlorinated compounds was published by a group of Occupational Health scientists at Harvard Medical School, led by Cecil Drinker. Dr. Drinker was one of the foremost occupational health physicians in the world. The Harvard Library collection of Dr. Drinker's 250+ scientific publications (Harvard Library, 2018) contains the following biographical description of Dr. Drinker:

"Cecil Kent Drinker was Professor of Physiology and Dean of the Harvard School of Public Health whose research specialized in industrial medicine and hygiene. Drinker was a pioneer in industrial medicine, and established industrial hygiene and applied physiology as disciplines in preventive medicine and public health. He was an authority on the lymphatic system, tissue fluid exchange, blood circulation, industrial and work-related poisoning and hygiene, as well as methods of artificial respiration. Drinker was one of the first physicians to stress the importance of the respiratory tract as the route of absorption of toxic dust and fumes, and after he completed research on manganese inhalation, became one of the leading experts in treating manganese poisoning in the United States.....From 1915 to 1916, Drinker served as an instructor in the department of physiology at Johns Hopkins Medical School. In 1916 he returned to Boston to take a position as a faculty instructor at Harvard Medical School in Walter B. Cannon's physiology department. Two months later, Cannon was called into active military service, and Drinker was named acting head of the Department of Physiology until Cannon returned in 1918. Drinker was subsequently appointed Assistant Professor in 1918, Associate Professor in 1919, and Professor of Physiology in 1923, a post he held at Harvard Medical School and the Harvard School of Public Health until his retirement in 1948. From 1924 to 1935 Drinker was Assistant Dean of Harvard School of Public Health and, from 1935 to 1942, Dean of Harvard School of Public Health. During World War II, Drinker conducted respiratory physiological research for the United States armed forces, and contributed to the development of high-altitude oxygen masks and goggles for allied aviators....Drinker published 250 articles, textbooks, and reports during his career on topics including the circulatory system, lymphatic system, industrial hygiene, asphyxiation, and physiology. In 1954 he published the textbook Clinical Physiology of the Lungs. He was also instrumental in starting the Journal of Industrial Hygiene and Toxicology. For many years, Drinker, his wife, and his brother Philip were the editors of this publication."

One of the first clinical toxicology reports of PCBs and PCNs was initiated at the request of the manufacturer of the Halowax Corporation, because three workers died of liver failure. The case report by Drinker *et al.* (1937) reported on the nature of the illnesses and death, attributing the deaths to cirrhosis of the liver. The Halowax workers were exposed primarily to mixtures of chlorinated naphthalenes, with some exposures to

polychlorinated biphenyls. This episode of occupational exposures led to some of the first laboratory animal studies on the toxicology of these compounds. Drinker *et al.* (1937) describe a series of experiments in laboratory rats, where the animals were exposed by inhalation to the vapors of various different mixtures of chlorinated naphthalenes and PCBs (then referred to as 'chlorinated diphenyls'), with exposures conducted for 16 hr per day, 6 days per week for about 11 weeks (total exposure time of 1896 hrs). A subset of animals was evaluated for evidence of toxic effects at 6 weeks. They also conducted feeding studies using the same mixtures. Although none of the animals exhibited any external evidence of toxicity, histopathology after autopsy of the animals found evidence of mild to moderate liver damage. This was not observed with the lower chlorinated naphthalenes (tri and tetra) but was evident for higher chlorinated (penta and hexa) naphthalenes and for the PCB/PCT mixture, which was also relatively highly chlorinated (65% chlorine by weight).

Based on this initial animal study, Dr. Drinker concluded the following, regarding the question of whether these chemicals can be used safely in the workplace:

*"In the basis of these experiments and on many field determinations of different compounds in the air of workrooms, it [tested PCB mixture] appears safe and it is certainly easily attained, to ventilate so that the air breathed does not contain more than 0.5 mgm [milligrams]. per cu. m. of any of these compounds above trichloronaphthalene. In the case of the latter compound concentrations of 10.0 mgm [per cu. m.] are permissible. We know from many examinations in many different plants that such concentrations have been greatly exceeded during the past 20 years, and we are conscious of the fact that our rat exposures have been inexorably constant whereas human exposure is never so ordered. **Time and careful observation may change these opinions as to standards, but today we are convinced they [PCBs] are safe.** Impregnating tanks and other arrangements utilizing the chlorinated hydrocarbons are easy to hood and to safeguard. Compared with benzene, lead tetraethyl and many other compounds, these substances are very little toxic and operations employing them can be safeguarded. It may be argued that if possible trichloronaphthalene should be used, but this compound will cause acne and if employed very carelessly might do more. Furthermore, higher chlorination is often essential for practical reasons. The solution consists in thoroughly adequate ventilation plus good housekeeping around all wax containers. [emphasis added]"*

Thus, Drinker and his colleagues at Harvard Medical School – among the most highly respected occupational health scientists of the day- felt that all of the compounds studied, including PCBs, could be used safely in the workplace, with standard industrial hygiene practices of the time that include proper care and ventilation.

This report was followed by two more laboratory animal studies from the same group of investigators (Bennett *et al.*, 1938; Drinker, 1939), where some additional mixtures of chlorinated naphthalenes, chlorinated terphenols (referred to then as 'chlorinated diphenylbenzenes') and chlorinated biphenyls were used. The content of these mixtures was described in the Bennett *et al.* (1938) paper as follows:

- Compound A. A mixture of tri- and tetrachloronaphthalenes. Chlorine content 49.4%.
- Compound B. A mixture of tetra- and pentachloronaphthalenes. Chlorine content 56.4%.

- Compound C. A mixture of tetra- and pentachloronaphthalenes plus chlorinated diphenyl. Chlorine content 43%.¹⁴
- Compound D. A mixture of penta- and hexachloronaphthalenes. Chlorine content 62.6%.
- Compound E. A mixture of penta- and hexachloronaphthalenes. Chlorine content 62.6%.
- Compound F. A mixture of 90% penta- and hexa-chloronaphthalenes, plus 10 % chlorinated diphenyl. Chlorine content 63 %.¹⁵
- Compound G. Chlorinated diphenyl. Chlorine content 65%.¹⁶

It is important here to realize that the third paper from Dr. Drinker, published in 1939 (Drinker, 1939) titled "*Further observations on the possible systemic toxicity of certain of the chlorinated hydrocarbons with suggestions for permissible concentrations in the air of workroom*" makes a correction in the nature of 'Compound G' (listed as chlorinated diphenyl). In this third study, Drinker *et al.* conduct even more studies with more PCB mixtures, as illustrated by the table, below, cut and pasted from the 1939 paper.

A LIST OF 14 CHLORINATED HYDROCARBONS, WITH CHLORINE CONTENTS AND PERMISSIBLE LIMITS (IN MG./CU.M.) FOR THE AIR IN WORKROOMS*

COMPOUND	CHLORINE CONTENT	PERMISSIBLE LIMIT
	%	mg./ cu.m.
1. Trichloronaphthalene plus a trace of tetrachloronaphthalene. Tested upon rats by inhalation and by feeding.....	49.9	10.0
2. Tetra and pentachloronaphthalenes. Tested upon rats by inhalation and by feeding.....	56.4	1.0
3. Penta and hexachloronaphthalenes. Tested upon rats by inhalation and by feeding, and upon dogs by feeding alone.....	62.6	0.5
4. Tetra and pentachloronaphthalenes plus refined chlorinated diphenyl. Tested upon rats by feeding.....	43.5	0.5
5. 90% penta and hexachloronaphthalenes plus 10% chlorinated diphenyl benzene. Tested upon rats by inhalation and by feeding.....	63.0	0.5
6. Chlorinated diphenyl plus chlorinated diphenyl benzene. Tested upon rats by inhalation and by feeding.....	65.0	0.5
7. Chlorinated diphenyl oxide. Tested upon rats by inhalation.....	54.0	0.5
8. Chlorinated diphenyl oxide. Tested upon rats by inhalation.....	57.0	0.5
9. Chlorinated diphenyl. Tested upon rats by inhalation.....	50-55	0.5
10. Hexachlor diphenyl oxide plus 5% trichloronaphthalene. Tested upon rats by inhalation.....	50-55	0.5
11. Hexachloronaphthalene and crude chlorinated diphenyl. Tested upon rats by inhalation.....	Un- known	0.5
12. Special chlorinated naphthalene. Tested upon rats by inhalation.....	50-55	0.5
13. Chlorinated diphenyl. Tested upon rats by inhalation.....	63	10.0
14. Chlorinated diphenyl benzene. Tested upon rats by inhalation.....	60	0.5

* The analytical method and apparatus used routinely for field determinations is that described by Tebbens (*Trans J., 18, 204 (1937)*) and by Drinker *et al.* (*ibid., p. 283*).

¹⁴ Compound C contained chlorinated diphenyl (compound G, which was incorrectly identified in the original paper and was later corrected to have been a mixture of chlorinated diphenyl and chlorinated diphenyl benzene).

¹⁵ Compound F was a mixture containing chlorinated diphenyl, whose identity was almost certainly Compound G which was incorrectly identified in the original paper and was later corrected to have been a mixture of chlorinated diphenyl and chlorinated diphenyl benzene.

¹⁶ Compound G identity was corrected by Drinker *et al.* (1939). Compound G was incorrectly labeled in as polychlorinated diphenyl; it was actually a mixture of chlorinated diphenyl and chlorinated diphenyl benzene.

In the earlier 1938 paper, 'compound G' (presumed at the time to be relatively pure PCBs) was highly toxic to the liver (Bennett *et al.*, 1938). In the later 1939 paper Drinker tested other PCB preparations (test groups 13 and 14 above), he found them to be almost completely non-toxic (Drinker, 1939):

*"The sixth compound has been listed previously as chlorinated diphenyl. It contained 65% of chlorine and proved very destructive to the liver. **Later experiments with compound 13, which contained 68% or chlorine and which was also labelled chlorinated diphenyl, were a surprise to us since this second compound was almost non-toxic.** On inquiry it was found that Substance 6 [compound G in the 1938 paper] was in reality a mixture of chlorinated diphenyl and chlorinated diphenyl benzene and that number 13 was actual chlorinated diphenyl. We have no information as to whether this last compound [PCBs] lacks toxicity because it is not broken down in the body, but that would seem the probable explanation [emphasis added]."*

Thus, the presence of chlorinated diphenyl benzene in 'compound G' was responsible for the liver toxicity observed with what was presumed initially to be 'pure' PCBs in the Bennett *et al.* 1938 paper. The Drinker 1939 paper concludes that PCBs are the least toxic of all 14 mixtures tested. From this study it is apparent that the liver toxicity that was responsible for the deaths of 3 workers from 'yellow atrophy' (liver disease) was the result of the higher chlorinated naphthalenes plus the contaminant, chlorinated diphenyl benzene, (substance 6 in the Drinker 1939 study), rather than to PCBs. His recommendation for an occupational standard for PCBs with a chlorine content of 68% was 10 mg per cubic meter, 20 times higher (less toxic) than what was recommended for the higher chlorinated naphthalenes and the chlorinated diphenyl benzene. Test substance 9 in the table above is listed as 'chlorinated diphenyl' with a chlorine content of 50-55%, for which he recommended an occupational standard of 0.5 mg/m³, although no details are provided on the specific pathological results of compound 9, and there is no information on the contents of compound 9, except that it contained 50-55% chlorine. Dr. Drinker makes no mention of the results of compound 9 in his report to Monsanto (Drinker, 1938b).

The Monsanto-commissioned studies that Dr. Drinker conducted on PCBs were in response to a 'trigger'-reports of human illness in the workplace following exposure to a variety of substances. The studies were conducted for the specific purpose of identifying which of several possible mixtures could have been responsible for the deaths of three workers, and to then establish safe working conditions (allowable exposure levels) for the various compounds. Dr. Drinker provided a report to the Monsanto Company (Drinker, 1938b) where discusses the liver toxicity seen with various Monsanto products containing chlorinated biphenyls and naphthalenes, and diphenyl benzene, and offers the following conclusion:

*"In conclusion, #1268 [PCB1260], if handled with ordinary precautions as to ventilation, should be **entirely harmless to workmen.** While it cannot be given an absolutely clean bill as to health, it is preferable to #4465 [later found to be chlorinated terphenyls or 'chlorinated diphenyl benzene'] and #5460 [PCB1260 + chlorinated diphenylbenzene] [emphasis added]."*

Thus, at this point in history, Dr. Drinker clearly indicated to Monsanto that PCBs were practically non-toxic to workers.

Several other basic toxicology studies of PCBs were completed in the 1940's (Miller, 1944; von Wedel *et al.*, 1943) which confirmed using several different species that the liver and skin were the sole 'target organs' for PCB mixtures. Miller (1944) noted that there were significant species differences in susceptibility to the toxic effects in the liver, with guinea pigs being more sensitive than rats or rabbits.

Monsanto later obtained further toxicology studies from Dr. Joseph Treon, who was director of the Kettering Laboratory, Department of Preventive Medicine and Industrial Health, in the College of Medicine at the University of Cincinnati (Dr. Drinker retired from Harvard Medical School in 1949). The Kettering Laboratory was widely recognized as the leading academic research center for industrial toxicology at the time.

Monsanto sought out Dr. Treon to conduct some basic toxicology studies on Aroclor 1254, Aroclor 1242, and Pydraul. The Kettering laboratory completed many studies of various PCBs, using different techniques to 'volatilize' the PCBs for airborne exposures to rats, mice, guinea pigs and rabbits. The results demonstrated that, when heated to extraordinary temperatures that were capable of initiating chemical decomposition to toxic breakdown products, vapors of PCB1248 could be toxic to the lungs of rats (Treon *et al.*, 1953a).

However, studies which utilize vapors of PCBs that were not subjected to extreme temperatures were far less toxic. For example, in the June 22, 1955 report to Monsanto on the 'Toxicity of the Vapors of Aroclors 1242 and 1254', Treon *et al.* (1955b) stated the following:

"The purpose of this investigation was to determine the effects upon animals of various species, of moderately prolonged intermittent exposure to air bearing the vapor of either Aroclor 1242 or Aroclor 1254. This report deals with the results obtained in experiments in which, for periods ranging up to 4 months, animals were subjected to respiratory exposure to air containing the Aroclors in concentrations that approached saturation and so were greater than any likely to be encountered in industry. The animals failed to exhibit any signs of intoxication during or after the exposure to which they were subjected, and the numbers of deaths which occurred in the exposed animals were comparable to those which resulted from incidental causes in the control groups. Gross and microscopic examination of the tissues of the animals of both the exposed and the control groups disclosed the existence of certain abnormalities which did not differ significantly in their severity or frequency of occurrence among the test and control groups."

In another subsequent report (Treon *et al.*, 1955c) these experiments were repeated at a lower dose (1.5 vs. 8.56 µg/L), but for a much longer period of time (7 hrs/day for 150 days over a 214 day period, vs 7 hrs/day for 17 days over a 24 day period). Not surprisingly, the prolonged exposure did result in some evidence of mild liver toxicity compared to the apparent lack of any significant toxicity for the shorter period of exposure. Treon *et al.* (1955c) provided the following conclusions from these prolonged studies:

"No relevant gross pathological changes were observed in the animals, but microscopic lesions of a mild, nonspecific toxic type were found in the livers of some of the Guinea pigs and mice and in all of the rabbits and rats. Similar changes were also found in the renal tubules of rats. Although microscopic abnormalities of this type are often found in the tissues of "normal" animals, those observed in certain of the control animals were not as severe as they were found to be the corresponding species of animals that had been exposed to Aroclor 1254. The conclusion seems to be warranted, therefore, that prolonged

respiratory exposure to Aroclor 1254 is capable of causing some injury to tissues of susceptible animals under conditions in which atmospheric concentration of the material is on the order of 0.11 parts per million."

Treon's work related to Pydraul was a very detailed study at a very high level of exposure and for a duration that would not likely ever be encountered in the workplace. Although some toxicity was observed under these extreme experimental conditions, after thorough consideration of the dose and exposure period, Dr. Treon provided the following summary conclusions regarding the relative safety of Pydraul F-9, compared to Houghto-Safe 271:

"In the foregoing experiments animals have been subjected to exposure for more than 3 hours to concentrations of Pydraul F-9 which were several times higher than the highest concentration of Houghto-Safe 271 which occurred (for a few minutes) in the pump room of the U.S.S. Lake Champlain. The essentially harmless effects of such experimental exposure to Pydraul F-9 speak for themselves."

Subsequent to these studies, Pydraul F-9 was considered as a hydraulic fluid for use in US Navy submarines. The Navy told Monsanto that they had conducted some 'toxicology tests' of Pydraul F-9 and the results indicated to them that it might not be safe for use on submarines intended to be submerged for extended durations of time. Dr. Treon's studies reflected the safety of Pydraul F-9 under the proposed conditions of use. The results of the Navy's studies were not revealed to Monsanto at the time, and, therefore, Monsanto could not evaluate the conditions of the Navy tests or the results.

Thus, at this point in history (late-1930's- early 1950s), Monsanto was aware that occupational exposure to PCBs had the potential to cause skin lesions and liver damage among workers if exposures were high enough. But two of the most respected toxicologists in the world both provided assurances that PCBs could be used in industry safely if appropriate, and readily achievable, industrial hygiene practices were implemented. None of these animal toxicology studies suggested any significant toxicity beyond effects seen in the liver and skin, and this point was emphasized repeatedly in Dr. Drinker's published studies and his report to Monsanto. Attachment 1 provides a list of all of these studies and the dates that were performed by Monsanto during through the early 1970s.

In 1970, Monsanto began to voluntarily withdraw PCBs from the market, starting with plasticizer uses in 1970. By that time, Monsanto commissioned hundreds of toxicological tests on Aroclors and PCB products that included studies on acute skin irritation, subchronic and long-term inhalation studies, human dermal sensitization studies, acute lethality studies, chicken residue/reproduction studies, subchronic and chronic rat and canine studies, a three-generation rat study, developmental studies, genotoxicity studies, fish toxicity studies, and Monsanto had initiated two-year chronic studies. These studies were conducted using state-of-the-science techniques for the evaluation of potential safety hazards associated with industrial chemicals where the triggers for cancer studies (discussed below) were not met.

B. Charge 3: Specifically evaluate the state of toxicological testing for cancer in that time period (1930's on) and whether there were any statutory, regulatory or industrial standards that would have required Monsanto to conduct animal testing for cancer.

Sub-opinion: Consistent with the industrial toxicology practices of the time, Monsanto had no reason to conduct cancer studies in laboratory animals in the 1930's-60's because

1. PCBs were never intended for use as pharmaceuticals, food additives, or as pesticides used on food crops, and thus there were no regulatory requirements or industry standards, nor was there any expectation of widespread human exposures beyond the workplace, that would have compelled Monsanto to conduct animal studies for cancer;
2. There was never any evidence that occupational exposures to PCBs caused cancer in workers exposed to high levels;
3. There was nothing about the chemical structure of PCBs that would have suggested it might be carcinogenic, based on chemical knowledge at the time;
4. None of the early studies on the toxicology of PCBs, including Dr. Drinker's studies, provided any compelling evidence that would suggest PCBs might be carcinogenic, and none of the experts they relied upon ever suggested that PCBs should be tested for carcinogenic activity;
5. There were no industry-wide expectation or standard testing protocols for carcinogenicity testing of industrial chemicals until the 1960's-70's.

Because lifetime (2-year) animal studies of Aroclors performed in the late 1970's and 1980's found that some Aroclor mixtures increased the number of liver tumors in rats, it has been alleged that Monsanto should have conducted such lifetime carcinogenicity bioassays of PCBs in the 1930's and 40's. However, standard procedures for conducting lifetime bioassays for carcinogenicity for industrial chemicals were not developed until the mid-1960's, and even today are not required for industrial chemicals. Although the Delaney Clause of the Food, Drug and Cosmetic Act (FDCA), passed in 1958, provided an impetus for the development of such assays, standard protocols were not developed until the late 1960's. During the 40's, 50's and 60's, the only substances that would have been considered for such long-term animal bioassays would have been those that met one or more of several 'triggers':

1. The compound is intended for use as a food additive (FDCA)
2. The compound is intended for use as a pharmaceutical (FDCA)
3. The compound is used as a pesticide on agricultural products meant for human consumption (Federal Insecticide, Fungicide, and Rodenticide Act; FIFRA)
4. The product had been shown, or suspected, of causing cancer in the workplace, following occupational exposures or was structurally similar to known or suspected workplace carcinogens, and thus might be 'suspect' based on chemistry of the compound.

PCBs were used primarily in the electrical industry, both for the production of wire insulation and as an insulating oil in transformers and capacitors; their use was not associated with occupational cancers in Monsanto or GE workers (Benignus, 1970). They were not intended or marketed for use as food additives or as pharmaceuticals (e.g., Monsanto Chemical Company, 1940), or as pesticides intended for use on crops

(e.g., Monsanto Chemical Company; Papageorge, 1971). Although there were some patent applications and internal discussions within Monsanto about potential uses of PCBs in pesticide formulations or in food packaging, these were never acted on and no Monsanto-produced commercial PCB products ever met conditions 1-3 above (Attachment 1C provides a list of reviewed product bulletins, application bulletins, and guides). Thus, the first three triggers listed above would not have provided a reason for conducting a carcinogenicity test.

Although occupational monitoring of workers using chlorinated hydrocarbons, including PCBs, identified health concerns, including 3 fatalities, related to 'yellow atrophy', as discussed above, these were thoroughly investigated by Dr. Drinker and others and demonstrated to be from another class of compounds (chlorinated naphthalenes and diphenyl benzene), and not from PCBs. Indeed, careful studies of workers exposed to large amounts of PCBs have failed to find a causal connection between occupational exposures to relatively high doses of PCBs and increased cancer (reviewed in Golden and Kimbrough (2009); see also Defendant's epidemiology Expert Report by Dr. Shields). Further, there was no evidence from any workplace that PCBs represented a cancer risk to workers.

1. History of the development of animal bioassays for cancer testing

The recognition that some substances are capable of causing cancer in humans dates back to Sir Percivall Pott's description of scrotal cancer in chimney sweeps, first reported in 1775 (Brown and Thornton, 1957). The first animal study to demonstrate that the complex mixture of chemicals in coal tar ('soot') was capable of causing cancer was completed in 1918 (Yamagiwa and Ichikawa, 1918), verifying that coal tar was responsible for the occupational cancers in chimney sweeps first identified by Pott. However, it wasn't until the 1930s that the specific chemicals presence in coal tar responsible for causing cancer were identified by the pioneering work of Kennaway (1930) and Cook *et al.* (1933).

Hueper *et al.* (1938) were the first to successfully demonstrate that bladder tumors, observed for several decades to be elevated in workers in the dye and textile industries, could be induced by implanting pellets of beta-naphthylamine into the bladders of dogs. Indeed, it is quite evident in reading the article that the purpose of the study was to reproduce a tumor already known to occur in the occupational setting, in laboratory animals for the purpose of identifying a blood marker that could be used to identify workers who might have the tumors in an early stage. The authors note the specific purpose of this study:

"Investigations of the chemical nature of the substances responsible for the production of the so-called 'aniline tumors' of the urinary bladder, and of the manner of their causative action have been seriously handicapped in the past by the fact that, so far, all attempts to reproduce these neoplasms experimentally have essentially been unsuccessful....The investigations to be reported were undertaken for two reasons: first, to provide, through experimental production of 'aniline tumors' in animals, a sound foundation for a future intelligent approach to the study of the various aspects of these occupational neoplasms; second, to obtain by periodical examinations of the blood of the animals used in these experiments, some information in regard to any possible systemic effect of prolonged exposure to one of the suspected carcinogenic aromatic amines (beta-naphthylamine) upon certain constituents of the blood which may have some relation to the development

of the tumors in the bladder and which may thereby be of diagnostic significance as premonitory symptoms."

Hueper *et al.* (1938) specifically note the repeated failure of laboratory animal studies using rats to reproduce this tumor, and the experiment that he conducted involved surgically implanting pellets of beta-naphthylamine into the bladders of dogs – hardly a ‘routine bioassay’ for testing unknown chemicals for their cancer-causing potential.

Although there were a variety of studies in laboratory animals in the late 1930s that looked at cancer as an outcome, there were no specific, accepted protocols in use at that time to test the potential carcinogenic activity of a chemical. Johnathan Hartwell, a research fellow at the National Cancer Institute, published a “*Survey of Compounds Which Have Been Tested for Carcinogenic Activity*” in 1941 (Hartwell, 1941). Although this ‘compendium’ listed nearly 1,000 different studies (all completed before 1939) on 696 different chemical compounds, it is striking how relatively few different classes of compounds had been studied for a period of time adequate to see tumors develop: there were only 13 studies on 9 different substances that had oral (feeding) exposures for more than 18 months (the ‘standard’ accepted exposure period for rodent carcinogenesis bioassays is 24 months, established in the early 1960’s by the NCI). Importantly, ALL 9 of these chemicals were already known or suspected human carcinogens: arsenic, alcohol (ethanol), beta-naphthylamine (an aromatic amine), dibenzanthracene (a polyaromatic hydrocarbon), o-aminoazotoluene (an azo dye), p-dimethylaminoazobenzene (an azo dye), scarlet red (an azo dye), and two endogenous substances, cholesterol, and estrogen. Equally striking is the wide variety of species used (6 used rats, 3 used mice, 2 used dogs, and one each used rabbit and ‘fowl’). None of these studies used ‘control’ groups, as the purpose generally was NOT to test to see whether the compound might cause cancer, but rather to try to understand better the process of chemical carcinogenesis, and/or to identify which specific chemical in a complex mixture might be the ‘culprit’ in causing cancer in the workplace environment. In terms of the number of animals used in a group, of the 13 oral studies that were 18 months or longer, 3 failed to indicate how many animals were used, 5 used 16 or fewer, 3 used between 20 and 30, and 2 used 100 or more. The Hartwell compendium thus does an excellent job of demonstrating that ‘carcinogenesis bioassays’ for the purpose of identifying whether an industrial chemical is carcinogenic or not simply did not exist at that time (1940). There were no standard protocols, and the nature of histopathological examination of tissues was crude, at best. In fact, this situation had not substantially improved over the next 15+ years. The use of chemical structure to predict carcinogenic properties was, and still is, very challenging, and fraught with uncertainty. This is well illustrated by the Discussion section of the first Hartwell compendium (Hartwell, 1941):

In this bulletin, data are collected on 696 different chemical compounds. Of these, 169 are reported to be carcinogenic, apart from 23 which are said to cause only papillomas. It is impressive, as has been pointed out by other writers, that these active compounds are found in a great variety of chemical classes, that substances of widely differing chemical structure possess similar carcinogenic activity; whereas of substances of the closest chemical similarity, one may be active and another completely inactive. These considerations may at first thought make it seem hopeless to connect carcinogenicity in any way with chemical structure. When 27 percent of all compounds tested have tumorigenic power, it would at first appear that there is little significance in chemical structure and that, while much light has been thrown on the origins of occupational cancer, a study of molecular architecture would not be expected to contribute in high degree to an understanding of the causation of spontaneous cancer.

However, more careful consideration brings one to the conclusion that we are far from justified in relaxing our efforts in this line of attack.

It is necessary to have a wide range of information in order to designate a compound as carcinogenic or noncarcinogenic. The carcinogenicity of a substance is known to be influenced by many factors² including the genetic constitution of the animal (species and strain), its age and sex, the diet, the physical condition of the animal, the purity of the chemical compound, the dose, the physical state of the compound, the nature of the solvent or vehicle used in administration, and the route or site of application. In addition, the value of the results is dependent on the number of animals used, the survival rate, and the duration of the experiment. Thus, the appearance of tumors is dependent to a high degree on experimental conditions, and both the number of tumors and the rate of their appearance are subject to many modifying influences. Furthermore, while failure to obtain tumors in a given case may be attributed to conditions of the experiment and should not always be taken to indicate lack of carcinogenic potency, the reports of tumors obtained should also be subjected to scrutiny and not necessarily accepted as proof of such potency. Many tumors are reported with no histologic support of malignancy; many are also reported as caused by the compound under test when only a few tumors are obtained in animal strains of unknown incidence of spontaneous tumors.

In a supplement to the 1951 Hartwell compendium, published in 1957 (Shubik and Hartwell, 1957), the authors make the following comment about the state of toxicity testing for carcinogenesis at that time:

This survey does not, unfortunately, provide the means for determining the likelihood of a compound tested routinely being carcinogenic. The majority of tests performed as toxicity investigations of new material are inadequate from the standpoint of carcinogenesis: many of these studies are not carried on for a long enough period, they often do not record pathological information in sufficient detail, and the bulk of these studies are confined to feeding experiments with rats, often of strains with such spontaneous tumor incidence as to make the detection of carcinogenicity quite impossible.

Although some academic and industrial laboratories in the 1940's and 50's began to develop their own protocols to test a chemical in laboratory animals for potential carcinogenic potency, it wasn't until the early 1960's that efforts began to develop standardized, 'predictive' animal bioassays using standardized protocols. The impetus for this was, in part, the passage in 1958 of the so-called "Delaney clause" of the Food, Drug and Cosmetic Act, which stated that *"the Secretary of the Food and Drug Administration shall not approve for use in food any chemical additive found to induce cancer in man, or, after tests, found to induce cancer in animals."* The National Cancer Institute (NCI), established in 1937, was the primary organization that had the responsibility for conducting cancer bioassays in laboratory animals in the 1950's and '60's. John and Elizabeth Weisburger, carcinogenesis specialists at the NCI, were charged in 1961 with developing 'standardized' approaches to chronic carcinogenesis bioassays, which was first published in 1967 (Weisburger and Weisburger, 1967). Dr. Weisburger provides the following summary of the state of the science in the early 1960's (Weisburger, 1999):

"This writer, together with Elizabeth Weisburger, and under the leadership of Michael Shimkin, was charged in 1961 with establishing a systematic approach to test chemicals for carcinogenicity, the National Cancer Institute Bioassay Program [4]. The classic rodent bioassay procedures were employed, with certain refinements such as the use of the then newly commercially available specific-pathogen-free mice and rats. Standardized methods of chemical administration at several dose levels were developed. It seemed that occupational cancer in humans was caused by chronic exposure at the work place to high dose levels of specific chemicals such as tars, asbestos, benzo(a)pyrene or 4-aminobiphenyl. Thus, the procedures adopted involved preliminary toxicology studies to determine the maximally tolerated dose in small groups, usually of 5–10 rats and mice. Examination of the existing literature led to the selection of an inbred strain of rat, the F344 rat, developed originally by W. Dunning, and a hybrid mouse strain, the B6C3F1, utilized by Kotin and Falk in their pioneering studies of lung cancer in animal models. The existing data showed both rats and mice of these strains had a fairly low incidence of spontaneous tumors, but seemed sensitive to a few carcinogens for which the literature existed at that time. Since Huggins proved the value of Sprague–Dawley strain rats for the induction of mammary gland tumors efficiently and rapidly, this method and rat strain was utilized in some exploratory investigations to validate this model for carcinogen bioassays, and also determine its limitations."

Finally, as noted by Hartwell in 1941, there was a substantial amount of research on the specific chemical structures required for carcinogenicity of then known occupational carcinogens, which included three main groups of chemicals: 1) polyaromatic hydrocarbons (PAHs, the substances present in soot and coal tar), 2) aromatic amines, used in a variety of industries, and 3) azo compounds, used primarily in the dye industry. Extensive 'structure activity relationship' data was developed in the 1920s and 30s on the PAHs. Indeed, in the Hartwell compendium, the vast majority of studies were on a few different PAHs, such as 1,2,5,6-dibenzanthracene. Studies of this compound alone constituted nearly 50% of all of the entries of studies conducted for 18 months or more by any route of administration. Of the 695 different chemical substances listed in the 1941 Hartwell compendium, over 350 were various PAH structures. From this extensive data in

the 1930s, there was a remarkably good understanding of the structural requirements for carcinogenic response to PAHs.

It is important to recognize that there was no requirement or expectation that 'industrial chemicals' (those chemicals not intended for use as food additives, pharmaceuticals or pesticides used on food crops) be tested in animals for their potential to cause cancer. Indeed, there is no such requirement even today, although the Toxic Substances Control Act of 1976 provided EPA with "authority to require reporting, record-keeping and testing requirements, and restrictions relating to chemical substances and/or mixtures." Testing requirements could conceivably include a requirement for a 2-year cancer bioassay, although this has seldom been done. Thus, given the absence of any of the triggers noted above, and the complete absence of any indication from workplace monitoring that PCBs had increased cancer risk among workers, Monsanto had no reason to conduct a 2-year carcinogenesis bioassay. In fact, they were not under any obligation to do so when they contracted with IBT in the late 1960's to conduct the first ever 2-year rodent carcinogenicity study with Aroclors, which did not demonstrate carcinogenic activity in that study.

Examples of other industrial chemicals produced by other manufacturers tell the same story (i.e., no carcinogenicity testing was performed in the lack of the triggers outlined above). Dow and DuPont were primary producers of chlorinated solvents such as trichloroethylene (TCE) and perchloroethylene (PCE or tetrachloroethylene), widely used in a variety of industries, including the dry-cleaning industries (Doherty, 2000a, b). In spite of being widely used with high-levels of exposure because of their volatility, these chemicals were not tested for their carcinogenic potential by either Dow nor DuPont (or any of the other manufactures of TCE and PCE). Even though manufacture and use of TCE started in the early 1930s, neither the Hartwell 1951 nor the Shubik and Hartwell 1957 compendiums list any long-term feeding studies related to trichloroethylene and TCE is not listed in the Hartwell 1941 compendium (Hartwell, 1941, 1951; Shubik and Hartwell, 1957). The first IARC Monograph on carcinogens, published in 1972, included entries for carbon tetrachloride and chloroform, but nothing on trichloroethylene (IARC and World Health Organization, 1972). An early review of TCE toxicity was provided by Shubik and Hartwell (1957). Numerous acute and sub-chronic studies of TCE were cited, including subchronic exposures at Dow chemical company laboratories Adams *et al.* (1951) as cited by Shubik and Hartwell (1957). But Dow never performed a 2-year chronic bioassay on TCE. The first study to demonstrate the possible animal carcinogenicity of TCE was conducted at the National Cancer Institute and reported in 1976, which is reported as the 2nd Technical Report from the National Toxicology Program (NTP, 1976). TCE was tested in the standardized NCI 2-year carcinogenesis study protocol, and was found to induce hepatocellular carcinomas in mice, but not in rats. However, because of the uncertainty over the rat data, TCE was again subjected to standardized carcinogenesis bioassays at the NTP in 1988, using 4 different strains of rats (NTP, 1988). Even this study, which was largely negative for tumors, was deemed inadequate:

"Conclusions: Under the conditions of these 2-year gavage studies of trichloroethylene in male and female ACI, August, Marshall, and Osborne-Mendel rats, trichloroethylene administration caused renal tubular cell cytomegaly and toxic nephropathy in both sexes of the four strains. However, these are considered to be inadequate studies of carcinogenic activity because of chemically induced toxicity, reduced survival, and deficiencies in the conduct of the studies. Despite these limitations, tubular cell neoplasms of the kidney were observed in rats exposed to trichloroethylene and interstitial cell neoplasms of the testis*

were observed in Marshall rats exposed to trichloroethylene" [*refers to original document and NTP ranking categories].

This is a good representation of exactly how challenging conducting a high quality 2-year bioassay can be, and why it was not, nor is it today, 'standard practice' to conduct 2-year bioassays on every new chemical to be used in commerce.

Similarly, PCE was one of the first chemicals selected for testing in the National Cancer Institute's Carcinogenesis bioassay program. The NCI report on PCE, published in 1977 (NTP, 1977), notes the following about early testing of PCE:

"Tetrachloroethylene [also known as perchloroethylene or PCE] (NCI No. C04580) is one of a group of halogenated organic solvents selected by the National Cancer Institute (NCI) for inclusion in the Carcinogenesis Bioassay Program. These solvents were selected on the basis of large-scale production, extensive use, and lack of adequate chronic toxicity data..."
"Human exposure to tetrachloroethylene is extensive. Approximately 85 percent of the compound consumed is used in a dispersive manner. The greatest human exposure takes place in dry-cleaning establishments using tetrachloroethylene, especially when ventilation is inadequate. Tetrachloroethylene appears to be a widespread environmental contaminant, found in air, water, and food. Worldwide air emissions of tetrachloroethylene were estimated at nearly 2.8×10^5 tons in 1974, and atmospheric concentrations normally range between 1 and 10 ng/liter."

As noted previously, the Shubik and Hartwell (1957) compendium had no entries for 2-year bioassays on PCE by Dow, DuPont or any of the other manufacturers of PCE, in spite of its extensive use in a wide variety of industries, with very large known exposures. The studies that were done, were all inhalation studies that were short-term (six months or less for rats and rabbits; less than eight months for monkeys and guinea pigs), with only one dose group, and small number of animals. In none of these inhalation studies were tumors found. This is because it was not standard practice in the 30s, 40s, 50s or 60s to test industrial chemicals for carcinogenesis in 2-year bioassays, unless the triggers I mentioned were met.

Despite the fact that tumors were not observed in any of the early inhalation studies, the results of the NCI 2-year rodent bioassay on PCE (NTP, 1977) found: *"In both male and female mice, administration of tetrachloroethylene was associated with a significantly increased incidence of hepatocellular carcinoma."* This provides another example of the fact that 2-year rodent bioassays were not industry standard even for high volume industrial chemicals where human exposure was expected to be high.

As shown above, 2-year bioassays on industrial products were not conducted because it was NOT standard practice to do so. In addition to TCE and PCE, Dow was a primary manufacturer of vinyl chloride (VC), which was found in the late 1970's to have caused a rare form of liver cancer in humans occupationally exposed to VC. V. K. Rowe, the director of the Dow Toxicology Laboratory for decades, discussed this in a 1975 article in the Annals of the New York Academy of titled "Experience in industrial exposure control" (Rowe, 1975). In this article, he makes the following comments:

"In the early days of Dow experience with vinyl chloride, from 1946-1959, relatively few samples of the workroom air were taken because no one was particularly concerned about

the toxicity of the material..... In 1959, when the results of our toxicological studies on animals caused us to be more concerned for both vinyl and vinylidene chloride exposure, continuous monitors were installed in our polymer plants and a rather dramatic reduction in workroom air concentration was achieved..... In the late 1960's, after discovery of acroosteolysis in workers in polyvinyl chloride operations abroad and in the United States, our workers were examined and no such disease was found. Consequently, we felt that our operations were generally acceptable. However, after Viola and co-workers' [1974] disclosed that they had induced tumors in rats exposed to high concentrations of vinyl chloride monomer, we decided to take a closer look at all of our operations. In addition to extensive medical surveillance programs, extensive industrial hygiene surveys were undertaken to assess in more detail the extent of our worker exposure to vinyl chloride."

C. Charge 4: Consider whether, if an animal test for cancer had been performed in the 1930's - 60's, it would have demonstrated that PCBs cause cancer in laboratory animals.

Had Monsanto found a laboratory willing and able to conduct a 'test for cancer' in the 1930s, '40s, or 50s, or established its own, it is highly unlikely that the study would have found a statistically significant increase in tumors from PCBs.

There are four primary reasons why this is true:

1. They likely would have tested the animals for 18 months or less, as was typical of such studies of that time. The 'positive' studies on Aroclors done in the 1980s-90s demonstrated unequivocally that the liver tumors that developed (largely in female rats) were slow to develop and generally not seen until near the end of the 2-year study (Mayes *et al.*, 1998; Norback and Weltman, 1985; etc., reviewed in Appendix 3).
2. They likely would have used an insufficient dose and/or route of exposure. Nearly all of the toxicology studies done on PCBs in 1930s-1950s were focused on workplace concerns about toxicity following inhalation exposure. The pioneering work of Drs. Drinker and Treon never really considered ingestion as the principle 'route of exposure', although they did do some feeding studies that corroborated the liver as the primary 'target organ' for toxicity of the mixtures of industrial compounds they were studying. Rather, their focus was, appropriately at the time, on workplace exposure via the inhalation route. Had Dr. Drinker or others conducted a 2-year inhalation study of PCBs, it is extraordinarily unlikely that it would have produced an adequate dose to the liver to cause liver tumors, which is the primary form of reproducible, statistically significant tumor develop in rat bioassays of PCB mixtures. For example, the Drinker study of PCB mixture #1268 (Drinker, 1938b) used two airborne concentrations, 0.58 mg/m³ for 16 hrs/day for 119 days, and then 6.23 mg/m³ for 16hrs/day for an additional 87 days. Using a rat respiratory rate of 100 breaths/minute, and a tidal volume of 1 ml/breath (Grant, 2014), the low dose exposure was approximately equivalent to dietary exposure at 10 ppm (assuming 15 gm per day of food consumption for a 300 gm rat), and the high dose exposure is roughly equivalent to 100 ppm of PCBs in the diet. As Drinker referred to the high dose as 'extreme conditions', it is highly unlikely that he or anyone else would have used that high of dose for an entire 2-year study. Further, the dose to the liver, where the tumors occur, is very much

lower following inhalation, rather than oral, exposure because of what is referred to as 'hepatic first pass clearance.' When PCBs and other highly fat-soluble substances are ingested, they must first pass through the liver. The liver is highly effective at removing and concentrating PCBs, before they are distributed to the rest of the body. In contrast, PCBs introduced into the body via inhalation are widely distributed throughout the body, resulting in lower concentrations in the liver, relative to what would occur following oral ingestion. Thus, even if a sensitive strain (Sprague-Dawley) of female rats was used, the concentration of PCBs in the liver following inhalation exposure would have been substantially less than that which occurs from feeding, and would not have been high enough to cause liver tumors, the only consistent type of tumor that has been established for PCBs in long-term feeding studies in rats (See Appendix 3 for a review).

3. Had Monsanto decided to conduct an inhalation test for cancer, it would likely have used a laboratory such as Dr. Treon's laboratory. Indeed, Dr. Treon's lab performed 8 such studies of industrial chemicals, listed in the Shubik and Hartwell 1957 Addendum. Attachment 2 shows these studies, which were representative of the type of inhalation studies used at the time. What becomes most obvious from an inspection of the Shubik and Hartwell addendum of Treon's inhalation studies is:
 - a) Nearly all studies were performed over a period of no more than 26 weeks, an insufficient time to see tumors develop
 - b) The maximum number of animals used in any study was 9, and most of the studies had 4 or fewer. Of the 9 studies that used rats, and reported the number of animals, 1 used 4 rats, 1 used 3 rats, and the remainder used 2 rats. The small number of animals used almost ensures that no significant increase in tumors would be seen;
 - c) A variety of species were used (guinea pigs, rabbits, rats, monkeys, cats)
 - d) In all of the 8 Treon inhalation studies listed in the (Shubik and Hartwell, 1957) addendum, they found a sum total of 0 tumors. Not a single study revealed any tumors. This is not surprising, for all of the reasons mentioned above.

Even when other studies of compounds suspected of being carcinogenic were conducted for 2 years using dietary administration, many of the completed studies failed to find a significant increase in malignant tumors. Hartwell notes in his 1941 compendium:

The number of entries in the present publication indicates an increase in the number of chronic toxicity tests for the period surveyed (1948 thru 1953) as compared with previous compilations. Out of 981 compounds listed, 779 are reported for the first time in these tests. Of the approximately 189 compounds in this Supplement which are found to induce tumors, 159 are new while 30 were reported as negative in the last edition of the "Survey". It can be seen that the proportion of carcinogens among the new compounds (21%) is similar to that found before (23%).

Thus, even when testing compounds for which there was already strong evidence that they were carcinogenic, over 75% tested negative. Again, this is simply a reflection of the state of the science for carcinogenic testing in this period of time.

4. Of 27 different 2-year studies on various commercial mixtures of PCBs (some using only males or females, some using both sexes; see Table 5 on p. 31, see also Appendix 3), only 7 of the 27 studies (26%) identified a positive response for cancers (and two others had increases in benign adenomas). Almost all of the increase in tumors that were seen were liver tumors and the response was largely in females. There are also obvious strain differences in rats, with female Sprague-Dawley rats showing, by far, the most sensitive response. This rat strain was not widely used prior to the protocol development effort of the Weisburgers in the early 1960s. For example, in a 1951 update to the 1941 Hartwell compendium (all of the studies listed in that compendium were indexed by species), of approximately 200 studies using 32 different strains of rats, only 13 used Sprague-Dawley rats. The most widely used strain was the relatively resistant Wistar rat, which was used in 40% of the studies.

The NCI study of Aroclor 1254 in 1978 was statistically 'negative'

It is important to note that the first three 2-year bioassays of PCBs, completed in 1971, 1972 and 1974, ALL failed to conclude that PCBs caused cancer in laboratory rats. The NCI's carcinogenicity of Aroclor 1254 also failed to show a statistically increased incidence of cancer in rats (NCI, 1978). Earlier studies (prior to 1970s) were limited due to the lack of standardized experimental animal cancer testing procedures (see Table 13 for differences before and after 1970).

Taken together, for all of the reasons discussed above, it is my opinion that, had Monsanto conducted a carcinogenicity study in the 1940s-1960s, using the tools available at the time, they would not have found PCBs to be carcinogenic.

IV. Comments on plaintiffs' expert report

A. Olson

1. General comments:

Dr. Olson's report spends a great deal of time discussing the adverse effects of high doses of PCBs to experimental animals. However, never once in the entire document does he address the single most important consideration in toxicology for evaluating relative risk, or 'safety' of consuming fish in the Spokane River – that of dose. He has provided no indication as to the quantity of fish that might be reasonably consumed by people that fish in the Spokane River. He has provided no indication that he understands what the concentrations of total PCBs, and specific congeners of toxicological interest, are in fish in the Spokane River. Thus, he has no idea what exposures are to various PCBs that might occur from consuming fish harvested from the Spokane River, and thus no idea of what a daily or lifetime dose might be. In the absence of ANY consideration of dose, it is impossible to make any scientifically based judgement about the relative harm that might come from consuming fish from the Spokane River, or the relative magnitude of public health benefit that might come from spending hundreds of millions of dollars attempting to lower the levels of PCBs currently present in the Spokane River. Thus, overall, his report is completely devoid of any useful toxicological information that could assist a judge and jury in determining the magnitude of harm, and thus harm reduction, that might be gained by expanding efforts to reduce the levels of PCBs in the Spokane River. Because, as I have set forward in my report, there is no meaningful risk associated with the levels of PCBs found in edible fish in the Spokane River, the levels of PCBs in edible fish in the the Spokane River do not make them unsafe for human consumption.

2. Specific comments:

Dr. Olson repeatedly fails to contextualize literature he cites to support his incorrect statements that "PCBs were known to cause adverse health effects as early as the 1930s and 1940s" and "studies in the 1950s further demonstrate the toxic effects of PCBs" on pp. 5-8 of his expert report. Olson discusses a publication by Jones and Alden (1936) that describes cases of chloracne in a group of workers manufacturing PCBs (which were called chlorinated diphenyls in the publication):

"Jones and Alden (1936) are physicians from Atlanta that investigated an outbreak of acneform eruption occurring in a group of workers engaged in the manufacture of PCBs (then called chlorinated diphenyls). Within a period from late summer of 1932 to October 1933, 23 out of 24 men working in the manufacture of chlorinated diphenyl were reported to have acneform eruption on the face and body. This article by Jones and Alden was published in 1936 in the Archives of Dermatology and Syphilology."

Olson's description implies that the investigation by the physicians supported his contention that PCBs were known to cause health effects in the 1930s. However, the opposite is true: PCBs were not known to cause chloracne at the time of the publication. Olson simply reported the Jones and Alden case descriptions of the chloracne outbreak but failed to include any of Jones' and Alden's summarization of results whereby they attributed the skin condition to other chemicals. Specifically, Jones and Alden stated: "the heating and chlorination of these impurities probably resulted in the production of styrene di-chloride and

chlorethylbenzene, which, we believe, on contact with the skin produced the acneform eruption ... since ... **chlorinated di-phenyl, did not, either experimentally or actually, produce any cutaneous or sebaceous irritation**" [emphasis added] Jones and Alden (1936)."

Schwartz (1936) is another publication incompletely discussed by Dr. Olson. The Schwartz publication was an attempt to address a data insufficiency - because to date, "dermatitis in the manufacture of synthetic resins and from their use has not been frequently reported or studied." Dr. Schwartz states: "the most important of the resins, from a standpoint of volume used, and skin hazards, are the phenol-formaldehyde and the urea-formaldehyde and the coumaron resins" [note chlorinated waxes, obtained by chlorinated mineral oils, paraffin, naphthalene, and diphenyl did not make the list]. However, synthetic waxes were discussed in the same section because the "principal synthetic waxes manufactured in this country are chloronaphthalenes and the chloro diphenyls [PCBs]." The only hazards listed by Dr. Schwartz for chloro diphenyl production workers included exposure to benzol (i.e., benzene) and diphenyl fumes. Dr. Schwartz indicated that workers involved in chlorinating the diphenyl and redistilling the crude Arachlor (sic) were affected with acne-like conditions of the skin as were workers exposed to fumes of chloronaphthalenes (e.g., Halowax). But, because patch tests performed with Halowax and chloro diphenyls were negative for irritation, Schwartz indicated the skin lesions of workers "probably result from the mechanical plugging up of the follicles of the skin with the waxes as the fumes solidify on the skin. The chlorine present on the waxes may have an irritating effect on the plugged follicles and cause suppuration." It was unclear if other effects were due to chloro diphenyl, Halowax, or an impurity of these products. This data hardly supports "early knowledge of PCB toxicity" as claimed by Dr. Olson.

Dr. Olson also attributes a 1941 publication whose conclusion is that the skin of the rabbit is a useful experimental animal for studying the pathology of human chloracne (Adams *et al.*, 1941). However, Olson failed to acknowledge many compounds were attributed with a "reaction" (no further test-article specific description of effects is given) on rabbit skin, including chlorinated diphenyls, chlorinated naphthalenes, chlorinated diphenyloxides, crude chlorinated phenols, and petroleum oils. No information was provided beyond a list of compounds associated with "effects." Furthermore, the absence of information regarding the severity of the reaction, the number of animals in control or test group, or the identity of the test article (beyond chlorinated diphenyl), prevents any scientific conclusion regarding the propensity of any of the listed compounds (if they tested pure compounds) for dermal effects.

A study by Meigs *et al.* (1954) was used by Dr. Olson as evidence that studies in the 1950s failed to identify a level of exposure that did not elicit an adverse effect and made clear the insufficiency of the ACGIH 1 mg/m³ standard. The report by Meigs *et al.* (1954) described an outbreak of dermatitis that didn't start until after exposures of up to 19 months (additional case recognition occurred because there was an "especially careful examination of the skin of all exposed employees after discovery of the first case"). Aroclor, which had been used in the facility as a heat exchange material for supplying heat to a reaction chamber, leaked under certain conditions and resulting air concentrations of chlorinated diphenyls was about 0.1 mg/m³. However, as heating of PCBs in the presence of oxygen is known to cause formation of contaminants (e.g., polychlorinated dibenzofurans; PCDFs); these contaminants were likely formed and the cause of the chloracne observed in the workers. Because PCDFs were almost certainly formed from the heating of the Aroclor, results of this study cannot be attributed to PCB exposure alone.

Finally, although Olson discusses observations reported by Miller (1944), he fails to contextualize the doses that were given to the animals in which the effects were observed. For example, the feeding dose given to rats was 138 mg/day. In the chronic evaluation done by Mayes *et al.* (1998), feeding exposure concentrations were about 1 to 4 mg/day (assuming an average male S-D rat body weight of 0.523 kg; US EPA (1988)). Therefore concentrations in the Miller (1944) study were at least one if not two orders of magnitude higher than those selected to evaluate toxicity many decades later. The purpose of the Miller (1944) study was to evaluate hazard at extremely high exposure doses – beyond those experienced in workers. Indeed, some exposure scenarios induced death in treated animals. Given that the amount of exposure governs the toxicity – anything can be toxic with a high enough exposure concentration – extrapolation of these results to “indicate that PCBs ... produce systemic toxicity” (Olson report, p. 127) is not supported by the study data.

The absence of scientific data in the 1950s regarding PCB toxicity in humans was summarized by Von Oettingen (1955) with the statement:

There are no reports on human poisoning from exposure to chlorodiphenyl alone, although numerous poisonings have resulted from its mixture with chlorinated naphthalenes.

This analysis demonstrates Olson selectively extracted data from scientific publications to support his contentions and failed to relate the study context, conclusions, and relationships to generally accepted scientific methodologies, practices, and concepts. Contrary to Olson’s contention, scientific studies from the 1930s to 50s do not support that PCBs were known to cause adverse health effects during those time periods.

On p. 13 of Dr. Olson’s report, he provides a discussion of Yusho and Yu-Cheng diseases that resulted from exposure to rice oil that was inadvertently contaminated with PCBs from a leaking heat exchange fluid used in the manufacture of the rice oil. While Dr. Olson’s description of the poisoning episodes is generally accurate, he fails to mention that this exposure is largely irrelevant to exposure to PCBs found in fish in the Spokane River and elsewhere because the oil was heated to high temperatures in the presence of oxygen, creating a substantial amount of polychlorinated dibenzofurans (PCDFs), which are exceedingly potent activators of the AhR, including the human AhR. It is now known that virtually all of the toxic effects observed in this population was from exposure to the PCDFs and not to PCBs. This was understood in the 1970’s. It is interesting to note that the first edition of *Casarett and Doull’s Toxicology: The Basic Science of Poisons*, published in 1975 (Casarett and Doull, 1975), has one mention of PCBs in the entire 750 page book (p. 562):

*In Japan, a form of chloracne known as “Yusho” (rice oil) disease was found to be caused by chlorinated biphenyl (PCB) contamination of one lot of rice oil (Panel on Hazardous Trace Substances, 1972). According to the authors, **a contaminant in the PCB may be the toxic substance, rather than the PCB**” (emphasis added).*

Dr. Olson should know that the symptoms from Yusho and Yucheng disease have been unequivocally demonstrated to be from the dibenzofurans and not from the PCBs themselves. In a 2015 review of these two tragic situations, Mitoma *et al.* (2015) state the following:

“This incident is called Yusho, which means oil disease in Japanese, because it was caused by the ingestion of rice bran oil contaminated with Kanechlor-400, a Japanese commercial

brand of polychlorinated biphenyls (PCBs) that was used in the process of refining the oil. It was later found that the oil was contaminated not only with PCBs but also with various dioxins and dioxin-like compounds (hereafter simply referred to as dioxins): polychlorinated quarterphenyls (PCQs), polychlorinated dibenzo-p-dioxins (PCDDs), and polychlorinated dibenzofurans (PCDFs) generated by heat denaturation of PCB..... The gas chromatogram pattern of PCBs in the blood of patients showed a characteristic pattern, A or B pattern, which was quite different than that in the blood of people unaffected with Yusho..... From 1975 to 1977, high levels of PCDFs were detected in the tissues of Yusho patients as well as in the contaminated rice oil (Nagayama et al., 1976, 1977)..... The relative contribution of PCDF congeners to the total TEQ in the contaminated rice oil was 77% (Yao et al., 2002). The most important compound causing the symptoms of Yusho (Masuda and Yoshimura, 1984), 2,3,4,7,8-penta-chlorodibenzofuran (2,3,4,7,8-PeCDF), the latest TEF of which was estimated to be 0.3 (Van den Berg et al., 2006), was shown to contribute 58% to the total TEQ in the oil (Yao et al., 2002)..... The estimated levels of total PCDDs, PCDFs, non-ortho PCBs, and total TEQ, respectively, in the mothers with Yusho at the time of delivery were 30.8, 161.7, 5.2, and 46.1 times higher than those in the general population (Masuda et al., 2005), respectively.”

Note that the levels of dioxin-like PCBs were only 5-fold higher than the general population, and contributed a very small amount to the total TEQ, especially if the TEQ were adjusted using human-specific TEFs reported by Larsson et al. (2015). Thus, these two situations are vastly different from trace level exposure to PCBs found in fish in the Spokane River, and are completely irrelevant to this case.

Dr. Olson’s statement that “it is likely that PCDFs contributed in part to the toxicity observed in these patients.” Seems to infer that that majority of the toxicity seen in these two populations is from PCBs, rather than from dioxins and dibenzofurans, but there is a vast literature that suggests exactly the opposite- that these symptoms are almost entirely due to the extraordinarily high exposure to highly toxic dibenzofurans, and that the contribution of relevant PCB congeners (i.e., those congeners found in fish in the Spokane River) contributed a fleetingly small amount to the observed effects. For example, Tsukimori et al. (2013) calculated the contribution of the various PCDDs, PCDFs and DL-PCBs to the total TEQ exposure to Yusho mothers who gave birth (Table 14). They used the WHO 2005 TEF values. Using those values, which greatly overestimate the contribution of PCB126 to the total TEQ, they found that the mean total TEQ concentration in 10 babies with symptoms of Yusho (so called ‘Black baby’, Table 14) was 2719.7, of which 2.1% was from DL-PCBs, and 97.9% was from dioxins and dibenzofurans, using WHO 2005 TEFs. However, if the 2015 recommended human TEF of 0.003, rather than 0.1, was used for PCB126, and the 2015 value of 0 was used for the PCB169 TEF, the contribution of DL-PCBs to the total TEQ would be somewhat less than 0.04%, which is toxicologically irrelevant.

Table 14. Estimated TEQ exposure using WHO 2006 TEF values (from: Tsukimori *et al.*, 2013)

Variables	Half life ^{a,b} (years)	TEF ^c	Black baby (n = 10)				Non-black baby (n = 107)				Odds ratio ^d	(95% CI)	p Value ^e
			% >DL	TEQ concentration			% >DL	TEQ concentration					
				Mean	SD	Median		Mean	SD	Median			
Total levels													
Total PCDDs	7 ^a		100	736.5	526.5	647.0	100	243.0	275.9	139.0	21.5	(1.48–310.9)	0.03
Total PCDFs	7.7 ^a		100	1923.9	2273.8	1313.1	100	460.7	905.3	141.6	3.46	(1.07–11.2)	0.04
Total coplanar PCBs	14.6 ^a		100	57.6	30.4	45.6	100	31.8	29.7	27.1	24.0	(1.25–463.6)	0.04
Total PCDDs, PCDFs and coplanar PCBs TEQ	7.7 ^a		100	2719.7	2706.8	1969.1	100	761.7	1164.0	410.8	7.54	(1.27–44.9)	0.03
Congener levels													
PCDDs													
2,3,7,8-TetraCDD	6.2 ^b	1	70	87.2	60.8	96.9	54	33.6	47.1	14.0	6.55	(1.00–42.8)	0.05
1,2,3,7,8-PentaCDD	8.6 ^b	1	100	213.7	153.1	180.9	100	75.0	78.4	52.4	28.4	(1.48–544.0)	0.03
1,2,3,4,7,8-HexaCDD	8.4 ^b	0.1	70	5.4	3.7	5.4	62	2.8	3.5	1.6	7.83	(0.86–70.9)	0.07
1,2,3,6,7,8-HexaCDD	13.1 ^b	0.1	100	41.1	34.0	33.8	100	15.2	16.9	10.0	28.6	(1.67–489.9)	0.02
1,2,3,7,8,9-HexaCDD	8.5 ^b	0.1	100	9.0	5.0	8.4	79	4.2	4.8	2.2	10.7	(0.94–122.3)	0.06
1,2,3,4,6,7,8-HeptaCDD	6.6 ^b	0.01	100	21.8	13.6	19.7	100	9.3	10.2	5.5	17.4	(1.24–242.8)	0.03
OctaCDD	5.6 ^b	0.0003	100	19.2	13.7	16.6	100	7.5	9.2	3.6	21.1	(1.64–271.7)	0.02
PCDFs													
2,3,7,8-TetraCDF	0.4 ^b	0.1	40	NA			50	NA					NA
1,2,3,7,8-PentaCDF	0.9 ^b	0.03	30	NA			24	NA					NA
2,3,4,7,8-PentaCDF	7.7 ^a	0.3	100	1728.4	2032.4	1191.4	100	404.9	805.8	122.8	3.27	(1.06–10.0)	0.04
1,2,3,4,7,8-HexaCDF	5.1 ^a	0.1	100	689.9	988.9	355.4	90	153.6	350.9	28.5	3.31	(1.09–10.1)	0.04
1,2,3,6,7,8-HexaCDF	5.1 ^a	0.1	100	254.1	307.2	167.7	90	66.8	117.5	18.6	4.83	(1.13–20.6)	0.03
1,2,3,7,8,9-HexaCDF	1.1 ^b	0.1	0	NA			0	NA					NA
2,3,4,6,7,8-HexaCDF	2.4 ^b	0.1	0	NA			10	NA					NA
1,2,3,4,6,7,8-HeptaCDF	3.5 ^a	0.01	70	41.2	39.2	27.2	60	11.1	17.9	2.6	4.93	(1.09–22.2)	0.04
1,2,3,4,7,8,9-HeptaCDF	3.2 ^b	0.01	0	NA			3	NA					NA
OctaCDF	0.2 ^b	0.003	0	NA			4	NA					NA
Coplanar PCBs													
3,3',4,4'-TetraCB(#77)	14.6 ^a	0.0003	0	NA			1	NA					NA
3,4,4',5-TetraCB(#81)	14.6 ^a	0.0001	20	NA			8	NA					NA
3,3',4,4',5-PentaCB(#126)	14.6 ^a	0.1	100	32.5	19.3	27.0	99	20.3	20.4	13.9	14.9	(0.90–246.6)	0.06
3,3',4,4',5,5'-HexaCB(#169)	14.6 ^a	0.03	100	25.1	20.1	23.2	100	11.5	13.6	7.2	9.74	(1.25–75.9)	0.03

^a Half-life data are from Masuda (2001).^b Half-life data are from Liem and Theelen (1997).^c WHO 2005 toxic equivalent factor values (Van den Berg *et al.*, 2006).^d Odds ratio for a 10-fold increase in blood lipid level adjusted for age at delivery, gestational age at birth, birth weight, descendant sex, and consumption of fish (times per week).^e Calculations were performed when the detection rate in the sample was >50%. DL, detection limit; NA, not applicable; CDD, chlorinated dibenzo-p-dioxins; CDF, chlorinated dibenzofurans; CB, chlorinated biphenyls.

A 2016 follow-up study of Yusho patients again measured concentrations of all dioxins, dibenzofurans and dioxin-like PCBs in these patients (Todaka *et al.*, 2016). The following table shows the relative concentration of dioxins, dibenzofurans and DL-PCBs using both the outdated WHO rat TEF values (van den Berg *et al.*, 2006), and the newer human TEF values determined by Larsson *et al.* (2015). I've included the relatively high human TEF value of 0.06 for PCB81 that was determined recently by Shi *et al.* (2019).

Table 15. Estimated TEQ levels of Yusho patients

	TEQ based on 2005 Consensus TEFs	% of Total, 2005 Consensus TEFs	TEQ based on 2015 Consensus TEQs	% of Total, 2015 Consensus TEFs
Dioxins	24	18%	34	10%
Dibenzofurans	82	63%	307	90%
PCB81	0.0016	0.0012%	0.32	0.093%
PCB126	13	10%	0.39	0.11%
PCB169	8.4	6.4%	0	-
Mono-ortho PCBs	3.1	2.4%	0	-
Total DL-PCBs	24	19%	0.71	0.21%
Total TEQ	130	-	342	-

Thus, using the newly described consensus TEFs for the HUMAN AhR, it is evident that the contribution of DL-PCBs to the total TEQ in Yusho patients is exceedingly small (0.21%), and the vast majority (90%) of the toxic effects are due to the presence of chlorinated dibenzofurans.

Dr. Olson further discusses the important role of chlorinated dibenzofurans and their formation from PCBs by heating at high temperatures, but fails to note that measured levels of dioxins and dibenzofurans in fish in the Spokane River are relatively low (WA Dept of Ecology, 2014), and that plaintiffs have not alleged that these compounds are present in fish in the Spokane River at levels that would present a significant risk to consumers of those fish.

Indeed, as noted above, Dr. Olson has made no attempt whatsoever to address the issue of 'dose' of PCBs, or anything else, that might occur from consuming fish from the Spokane River.

Dr. Olson discusses the important concept of TEFs for converting DL-PCB concentrations to 'toxic equivalence' values (TEQ), and cites van den Berg *et al.* (2006) (Olson Expert Report, Oct. 11, 2019, p. 14). But he fails to note that these values represent the potency in rats, not humans. Van den Berg and colleagues (Larsson *et al.*, 2015) provide a thorough reevaluation of TEFs for both rat and human, and provide the following NEW TEF values that should be used in place of the outdated 2005 values:

Table 16. Consensus toxicity factors
(from: Larsson *et al.*, 2015)

Table 2. Consensus Toxicity Factors for Compounds with World Health Organization Toxic Equivalency Factors

compound ^a	CTF		WHO-TEF
	rat	human	
Chlorinated Dibenzo- <i>p</i> -dioxins			
2378-TCDD ^b	1	1	1
12378-PeCDD ^b	0.5	1	1
123478-HxCDD ^c	0.2	0.03	0.1
123678-HxCDD ^b	0.06	0.06	0.1
123789-HxCDD ^c	0.3	0.002	0.1
1234678-HpCDD ^b	0.04	0.2	0.01
OCDD ^c	— ^e	0.005	0.0003
Chlorinated Dibenzofurans			
2378-TCDF ^b	0.2	0.1	0.1
12378-PeCDF ^c	0.2	0.6 ^{ex}	0.03
23478-PeCDF ^b	0.2	1	0.3
123478-HxCDF ^b	0.09	1	0.1
234678-HxCDF ^b	0.07	0.06	0.1
123678-HxCDF ^c	0.07	0.04 ^{ex}	0.1
123789-HxCDF ^c	0.3	0.02	0.1
1234678-HpCDF ^b	0.01	0.01	0.01
1234789-HpCDF ^b	0.05	0.3	0.01
OCDF ^c	0.007 ^{ex}	0.2 ^{ex}	0.0003
Non- <i>ortho</i> -substituted PCBs			
PCB77 ^b	0.0004	— ^d	0.0001
PCB81 ^c	0.0002	— ^d	0.0003
PCB126 ^b	0.09	0.003	0.1
PCB169 ^b	0.002	— ^d	0.03
Mono- <i>ortho</i> -substituted PCBs			
PCB74 ^b	0.000004	— ^d	—
PCB105 ^b	0.00001	— ^d	0.00003
PCB114 ^c	0.00006	— ^d	0.00003
PCB118 ^b	0.000009	— ^d	0.00003
PCB123 ^c	0.000009	— ^d	0.00003
PCB156 ^b	0.00008	— ^d	0.00003
PCB157 ^c	0.00003	— ^d	0.00003
PCB167 ^b	0.000007	— ^d	0.00003
PCB189 ^b	0.000007	— ^d	0.00003

^aNames of compounds are abbreviated as listed in the Materials and Methods. ^bBased on condensed information (PCA) from the experimental *in vitro* REPs. ^cBased on predictions from QSAR models. ^dNo value reported due to the inactivity of PCBs in the human bioassays. ^eNo value reported because the compound's membership probability value in the model was too low (below 99% confidence). Predictions were made at the 99% confidence level (marked "ex") and at 95% confidence level (unmarked). The membership probability values are located in the Supporting Information (Table S7).

It is evident from the values in Table 16 that the contribution of dioxin-like PCBs present in environmental samples, including fish the Spokane River, to total human TEQ is remarkably small, relative to amounts that have been shown to cause the many toxic effects in laboratory animals Dr. Olson discusses in his report. Not only does he fail to acknowledge the Larsson *et al.* (2015) report, but he also fails to acknowledge ANY of the numerous other studies demonstrating that the human AhR is far less responsive to DL-PCBs than rats (for example: Budinsky *et al.*, 2010; Carlson *et al.*, 2009; Shi *et al.*, 2019; Silkworth *et al.*, 2005; van Ede *et al.*, 2016; Zeiger *et al.*, 2001). His failure to note this remarkable species difference between humans and rats makes his conclusions about the relevance of experimental studies in rats with PCB mixtures, and the discussion about the toxicology of dioxins and dibenzofurans, incomplete, inaccurate and misleading when it comes to assessing two of the primary issues in this case – 1) are the fish in the Spokane River safe to eat at current levels of PCBs found in fish?, and 2) what is the net public health benefit to be gained by efforts to reduce the levels of PCBs the Spokane River? He fails to address either of these issues directly, but rather discusses large volumes of toxicological studies that are largely irrelevant in the absence of any context of dose and relative human susceptibility to dioxin-like PCBs.

Comments on the mode of action of PCBs as carcinogens:

On pp. 16-17 Dr. Olson states that PCBs (both non-dioxin like and dioxin like) act as tumor promoters.

While this statement is likely to be true, at least at very high doses, he fails to discuss any of the extensive mechanistic literature provided by the National Toxicology Program to address exactly this issue – what is the mechanism/mode of action by which PCBs cause liver tumors in experimental rats (that occurs almost exclusively in female rats)? He does not discuss the large volume of scientific data, including the NTP studies, and the studies by Mayes *et al.* (1998) and Brown *et al.* (2007) that demonstrate convincingly that the primary – if not sole- mode of action of PCBs in causing liver tumors in rats is through downstream events following activation of the AhR (e.g., is mediated via dioxin-like PCBs). Indeed, the National Toxicology

Program conducted a carefully designed 2-year study with large doses of PCB153, the predominant non-dioxin-like PCB found in the environment. The conclusions of this study were that PCB153 was “Under the conditions of this 2-year gavage study there was equivocal evidence of carcinogenic activity of PCB153 in female Harlan Sprague-Dawley rats based on the occurrences of cholangioma of the liver.” The NTP studies with PCB126 and PCB118, both ‘dioxin-like’ PCBs found overwhelming evidence of carcinogenic effects of these dioxin-like PCBs, and this is discussed in detail in my report. Given the strong effects that non-dioxin-like PCBs have on hepatic microsomal enzymes, I do not dispute that, at doses sufficient to cause prolonged activation of microsomal enzymes via activation of CAR and PXR, non-dioxin-like PCBs likely do have some promotional effects in rat liver tumor development at high doses. However, the doses necessary for that type of effect, if it does exist, are thousands of times higher than could possibly be achieved by consuming fish from the Spokane River.

Olson states on p. 19: “Total TEQ contributions (ppm) in Aroclor 1016, 1242, 1254, and 1260 are 0.11, 7.8, 23.4 - 47.6, and 7.2, respectively (Mayes *et al.*, 1998). This is of importance since the carcinogenic activity of Aroclor 1016 can be attributed to non-dioxin like PCBs. The rat studies with Aroclor 1016 also support the IARC 2016 conclusion that that the carcinogenicity of PCBs cannot be solely attributed to the carcinogenicity of the dioxin-like PCBs.”

As noted earlier, the only treatment-related tumors seen in the Mayes *et al.* (1998) study for Aroclor 1016 were a small number of liver adenomas at the two highest doses (100 and 200 ppm), and possibly ONE hepatocellular carcinoma in females at a dose of ~11,000 micrograms Aroclor 1016 /kg/day. No treatment-related (above the control rate) increase in tumors (benign or malignant) in males was evident at any dose of Aroclor 1016.

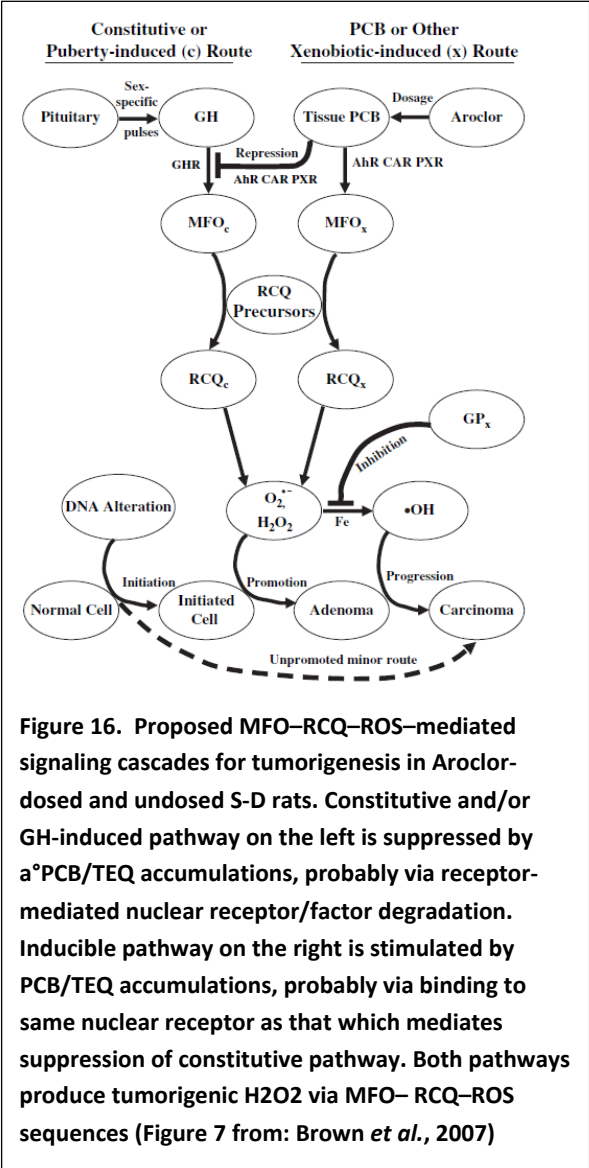
Table 17. Incidence of neoplastic liver lesions (Table 8 from: Mayes *et al.*, 1998)

Group	Dose (ppm)	Sex	Hepatocellular adenoma (%)	Hepatocellular carcinoma (%)	Hepatocholangioma (%)	Hepatocholangiocarcinoma (%)	First tumor ^b	Total animals with neoplasms (%)
Control ^a Aroclor 1016	0	M	4 (4)	3 (3)	0 (0)	0 (0)	91	7 (7)
	50	M	1 (2)	1 (2)	0 (0)	0 (0)	105	2 (4)
	100	M	1 (2)	1 (2)	0 (0)	0 (0)	105	2 (4)
	200	M	2 (4)	2 (4)	0 (0)	0 (0)	79	4 (8)
Control Aroclor 1016	0	F	1 (1)	0 (0)	0 (0)	0 (0)	105	1 (1)
	50	F	1 (2)	0 (0)	0 (0)	0 (0)	105	1 (2)
	100	F	5 (10) ^c	1 (2)	0 (0)	0 (0)	87	6 (12) ^d
	200	F	5 (10) ^c	0 (0)	0 (0)	0 (0)	63	5 (10) ^e

Olson claims that, because the TEQ for 1016 was 0.11, it must not have been AhR activity that contributed to this small increase in adenomas, and thus this provides evidence that non-dioxin-like PCBs caused this tumor. I agree that it is unlikely that the level of TEQ in the Aroclor 1016 is responsible for the 5 benign and 1 malignant liver tumor seen in the female rats, and that an alternative process was responsible. It is important to note that liver weight was statistically increased in females at both the 100 and 200 ppm doses of Aroclor 1016, indicating that there was extensive microsomal enzyme induction at these doses. Brown *et al.* (2007) evaluated the livers from the Mayes *et al.* (1998) study and found a striking relationship between the production of superoxide anions and the incidence of liver tumors across ALL Aroclors, including Aroclor

1016, in both males and females. The extensive induction of microsomal enzymes associated with the presence of AhR, CAR and PXR activating PCBs resulted in an increase in oxidation of circulating estrogen to form redox-cycling catechol estrogens, which were responsible for the oxidative-stress-induced liver tumors, as illustrated in Figure 16.

The point is that although it is possible at very high doses that non-Dioxin-like PCBs may contribute to tumorigenic activity in female rats via the same 'downstream' mechanisms of AhR activation (i.e., changes in oxidative stress through increased oxidation of estrogen to catechol estrogens) and whether this is mediated via CYP1A1, and AhR regulated microsomal enzymes, or CYPs 2B of 3A and other microsomal enzymes regulated by PXR and CAR, this mechanism of action would occur only at extraordinarily high doses of PCB mixtures (in fish). The formation of oxidative stress via catechol estrogen formation explains the high



selectivity of females for PCB-induced liver tumors. But, as discussed my report (Appendix 3), induction of microsomal enzymes from non-DL PCBs, which is mediated via CAR and PXR, is largely irrelevant to humans because even potent activators of human CAR, such as phenobarbital, are not carcinogenic in humans (Elcombe *et al.*, 2014), and PCB mixtures are not effective activators of human PXR (Tabb *et al.*, 2004). Further, the dose of total PCBs in the Aroclor 1016 studies (11 mg/kg/d at 200 ppm) was approximately 800,000 times greater than total PCB intake from consumption of sport fish from the Spokane River, using the most extreme reasonable upper bound assumptions for daily intake of Spokane River fish (i.e., 95% of consumption rate x mean concentration = intake of 0.0139 µg/kg/d, or 1.4 x 10⁻⁵ mg/kg-d). When using EPA-modeled scaling factors to convert daily intake to mg/m³ of body surface area (factor of 4.2 for rat to human conversion), the dose of Aroclor 1016 was still approximately 200,000 times greater than maximal human intake from Spokane River fish. Dr. Olson is a knowledgeable toxicologist, with full understanding of dose-response relationships, including those for microsomal enzyme induction. He knows that such curves are invariably steep, with changes from no measurable effect to maximal induction in the range of less than 100-fold. At the doses of PCBs that even the highest fish consumers would

get, the probability of ANY change in microsomal enzyme activity is zero. Dr. Olson also knows from extensive mechanistic data from animal carcinogenicity bioassays conducted by the National Toxicology Program that tumorigenesis of PCBs requires extensive and prolonged activation of microsomal enzymes, whether by AhR activation, or other nuclear receptors (e.g., CAR, PXR). It is simply toxicologically impossible for the doses of non-dioxin like PCBs found in fish in the Spokane River to cause prolonged and extensive microsomal enzyme induction in humans that would be necessary to cause significant oxidative stress that is required for even the very weak tumorigenic response seen in the rats receiving Aroclor 1016 in the Mayes *et al.* (1998) study.

In complete contrast to Dr. Olson's suppositions, the Mayes *et al.* (1998) study with various Aroclors, including Aroclor 1016, provides overwhelming mechanistic data to demonstrate that the rat liver tumor endpoint used by the EPA and the State of Washington in assessments of cancer risk from PCBs from consumption of fish are completely wrong because 1) they assume that risk is proportional to dose even at doses many orders of magnitude lower than the doses given to experimental animals (i.e., that there is no threshold for tumor response, even at many orders of magnitude lower dose); 2) the overwhelming scientific evidence demonstrates that humans are many orders of magnitude less sensitive to the induction of CYP1A1 and other microsomal enzymes mediated via the AhR because of dramatic species differences in the potency of AhR activation by 'dioxin-like' PCBs and 3) microsomal enzyme induction (via CAR and PXR) would not occur given the dose of 'non-dioxin-like' PCBs required to stimulate microsomal enzyme induction is thousands of times greater than maximum estimated doses that could be received via consumption of Spokane River fish. It stretches toxicological credulity to even suggest that non-DL PCBs in fish at the concentrations found in the Spokane River could pose a cancer risk to humans, based on the incredibly small response seen in rats given enormous doses of Aroclor 1016.

Dr. Olson also contends that there is evidence that PCBs are genotoxic (cause damage to DNA) and seems to imply that this could be an important 'mode of action' for the carcinogenic effects of PCBs found in the environment. He cites several studies showing positive mutagenic response from hydroxylated or PCBs with only one or two chlorines that are not abundant in Aroclors and/or do not bioaccumulate, so they are completely irrelevant to a risk evaluation of PCBs in fish in the Spokane River. He cites the study by (Liu *et al.*, 2017) demonstrating mutagenic activity of PCB20, PCB22, PCB52 and PCB74 in a highly artificial system where high levels of activating enzymes are present. Under those conditions, these PCBs did indeed appear to be mutagenic at 2-10 uM. What Dr. Olson failed to mention was the dozens of mutagenicity assays done in a wide variety of assays that do NOT find mutagenic activity of PCBs. In the Liu *et al.* (2017) paper he cites, the authors appropriately acknowledge the conflicting literature, stating:

"However, previous in vitro studies with PCB 52 provided inconsistent results. PCB 52 had provided negative results in reverse gene mutation assays in S. typhimurium with and without S9 (Wyndham et al. 1976; Hsia et al. 1978), sister chromatid exchange and chromosomal aberration tests in human lymphocytes in culture without S9 (Sargent et al. 1989) as well as chromosomal mutation tests in rats treated for 7–12 months with this PCB (Sargent et al. 1992; Meisner et al. 1992)."

But Dr. Olson chose not to make these discrepancies evident in his report.

If there are circumstances where PCBs found in the environment are capable of causing mutations or other forms of DNA damage, it is my opinion that the very weak effect, coupled with the extremely low concentrations, would not contribute significantly to the very large background of DNA damage that happens every day in every cell of our bodies. Fortunately, DNA repair processes are remarkably efficient at repairing such damage UNLESS the magnitude of the dose overwhelms DNA repair, such as occurs with chronic smoking, exposures to chemotherapeutic agents, and high doses of ionizing radiation. Dr. Olson's contention that genotoxic effects of PCBs could represent a significant mode of action for carcinogenic effects of PCBs observed in several animal studies is not supported by the vast majority of the literature that has addressed this question.

B. DeGrandchamp Books 1, 2, and 3, dated Oct. 11, 2019

1. Dr. DeGrandchamp's comparative pathology analysis (beginning on Dr. DeGrandchamp Expert Report, Book 1, Oct. 11, 2019, p. 39) of Dr. Bennett's tested mixtures is methodologically flawed and scientifically unreliable

Dr. DeGrandchamp's expert report includes discussions of the "Drinker studies" (Bennett *et al.*, 1938; Drinker, 1939; Drinker *et al.*, 1937). Following a methodologically flawed analysis of the Bennett *et al.* (1938) study, Dr. DeGrandchamp's conclusion that "*adding a small quantity of PCBs to the chlorinated naphthalenes caused an overall increase in the severity of liver pathology* (DeGrandchamp Expert Report, Oct. 11, 2019, p. 48)" is invalid because it relies on the erroneous interpretation that only PCBs were added to chlorinated naphthalenes. As explained below, although Bennett *et al.* (1938) initially described testing a compound identified as chlorinated diphenyl with 65% chlorine content [a PCB mixture], the authors later clarified that the material had been misidentified and was not a pure PCB mixture. The compound tested "*...was in reality a mixture of chlorinated diphenyl and chlorinated diphenyl benzene*" (Drinker, 1939).

The study design of the Bennett *et al.* (1938) paper involved testing several different chlorinated hydrocarbons individually and in mixtures. The specific test articles are provided in Table 18. Notably, Dr. DeGrandchamp's analysis failed to properly identify the evaluated compounds included in the mixtures. Specifically, Compound C, according to the footnote in Bennett *et al.* (1938) "*consisted of a mixture of compounds B and G but in addition contained two plasticizers which have been considered to be inert. Without these materials the chlorine content of this compound would be between that of compounds B and D.*" The footnote explains why the chlorine content of C did not match those of its components (because plasticizers had also been added). Compound F, according to the text of Bennett *et al.* (1938) was "*a mixture of 90 percent penta- and hexa-chloronaphthalenes, plus 10 per cent chlorinated diphenyl. Chlorine content 63 per cent.*" Therefore, compound F almost certainly consisted of 90% compound D and/or E (both listed the same composition) and 10% compound G. This is supported by the overall chlorine content of compound F, which matches the mixture composition and the failure to indicate any other source of chlorinated diphenyls for the experiments.

Table 18. Test articles evaluated by Bennett *et al.* (1938)

Compound	Mixture description	Chlorine content	Comment
A	Tri- & tetrachloronaphthalenes	49.4%	-
B	Tetra- & pentachloronaphthalenes	56.4%	Component of compound C
C	Tetra- & pentachloronaphthalenes, plus chlorinated diphenyl	43.5%	<i>Per Bennett et al. (1938)</i> : composed of Compound B, Compound G , and 2 plasticizers
D	Penta- & hexachloronaphthalenes	62.6%	Difference to Compound E not specified
E	Penta- & hexachloronaphthalenes	62.6%	Difference to Compound D not specified
F	90% penta- & hexa-chloronaphthalenes, plus 10% chlorinated diphenyl	63%	[90% Compound D and/or E and 10% Compound G] (<i>see text for explanation</i>)
G	Chlorinated diphenyl*	65.0%	*actually a mixture of chlorinated diphenyl and chlorinated diphenyl benzene, corrected in Drinker, 1939

Dr. DeGrandchamp acknowledged there was a “mix-up” with compounds tested by Bennett *et al.* (1938) whereby “Compound G. Chlorinated diphenyl. Chlorine content 65.0%” was later corrected to its actual composition, which was a mixture of PCBs and chlorinated diphenyl benzene (i.e., terphenyls). Rather than acknowledging the mislabeled test material and the introduction of terphenyl toxicity, Dr. DeGrandchamp “ignored all the experiments and discussions regarding Compound G” (DeGrandchamp Expert Report, Oct. 11, 2019, p. 40).

Dr. DeGrandchamp also failed to recognize that other mixtures used in his analysis (Compounds C and F) included the terphenyl-containing Compound G and were not pure mixtures of PCBs (as Dr. DeGrandchamp’s report indicates). Thus, by his own admission, all results related to Compounds C and F should have been ignored as any observed effects could not be attributed to PCBs (because the mixture also contained an unspecified amount of chlorinated diphenyl benzene [i.e., terphenyls]). Any and all conclusions regarding the inherent toxicity of PCBs based on effects observed following exposure to a mixture of PCBs and terphenyls, without knowledge of the mixture composition and effects induced by terphenyls, are scientifically invalid.

2. Dr. DeGrandchamp misrepresents that mitotic bodies and hyaline bodies were markers for early hallmark of tumorigenesis at the time of the Drinker and Bennett studies in the 1930s

Included in Dr. Drinker *et al.*’s (1937) description of the histopathology was mention of ‘many’, or ‘occasional’, mitotic figures in the livers of affected rats. DeGrandchamp’s claim that Dr. Drinker’s identification of mitotic figures in liver of PCB-treated rats was a ‘hallmark of early cancer’ is incorrect. Although Degrandchamp spends pages and pages discussing how mitotic figures are present in cancer, and how ‘abnormal mitotic figures’ are not always present in cancers, that discussion is irrelevant, often incorrect, and completely misses the point. Mitotic figures are numerous in tumors/cancerous growths and are used to identify margins of tumors, etc., because tumors, by definition, are clusters of rapidly dividing cells. But not all clusters of rapidly dividing cells are tumors, and the presence of excess mitotic figures alone has NO predictive value for carcinogenesis when observed in non-cancerous tissues like the liver that Dr.

Drinker evaluated. Indeed, scientific literature from that same time period indicate abnormal (aberrant) mitotic figures were not associated with cancer (Ludford, 1925; Mendelsohn, 1935).

The tissue that Dr. Drinker evaluated was NOT tumor tissue, it was liver tissue for which there was mild evidence of liver toxicity. As Drs. Bennett and Drinker specifically pointed out in their 1938 report, the presence of an increase in number of mitotic figures is nothing more than an indication that liver cells have been damaged, and that tissue regeneration (repair) was under way, as they indicated: *"The less damaged liver cells at the periphery of the lobules showed large numbers of mitotic figures, indicating accelerated regenerative activity"* (Bennett *et al.*, 1938, p. 104). There is a huge volume of literature on the remarkable ability of the liver to undergo regenerative repair, and it has been known for more than 100 years that damage to the liver is accompanied by an increase in regenerative cell division -e.g., there will be more mitotic figures in the liver following tissue injury. It has nothing to do with whether a chemical might cause cancer, or not. There is NO 'cancer predictive value' in whether mitotic figures are present in the liver following acute toxic injury, as occurred with the mixture of chlorinated naphthylenes and biphenyls being studied by Drinker *et al.* in 1938 and 39. My former University of Washington colleague and Chair of Pathology, the late Dr. Nelson Fausto, was among the world's experts in understanding the molecular process of liver regeneration after injury. The presence of increased numbers of mitotic figures IS a hallmark of liver regeneration in response to injury. It is NOT a hallmark or in any way a 'predictor of carcinogenic potential' when observed in non-cancerous liver tissue following liver injury, as claimed by DeGrandchamp. See for example, a few of Dr. Fausto's reviews (Fausto, 2004; Fausto *et al.*, 2012).

Although it was acknowledged that mitotic figures could be observed in malignant tumors in historical textbooks as early as 1938 (Boyd, 1938; Smith and Gault, 1938), it was because the number and type of mitotic figures (amongst other histologic criteria) were suggested indicators for estimating the degree of malignancy of an existing tumor (Smith and Gault, 1938). By 1953, Boyd stated the presence of mitotic figures within tumor tissue was "suggestive" of malignancy (Boyd, 1943, 1953).¹⁷ In 1961, Boyd further stated that mitotic figures were also seen in granulation tissue and in other rapidly regenerating cells and are "no proof of malignancy" (Boyd, 1961). It is important that in all of the early citations mitotic bodies were observed only in tumorous tissue and considered to be an indicator of malignant potential of tumorous tissue. There was no discussion in the textbooks regarding their presence in normal tissue as potential early hallmarks of tumorigenesis as Dr. DeGrandchamp indicated in his expert report.

In a similar manner, "hyaline degeneration," is not generally-accepted as a predictor of cancer in animals. A search of the National Toxicology Program database indicates that several chemicals have been tested for which it was concluded that there was insufficient evidence of cancer in liver despite the presence of "hyaline degeneration." Two examples are 2,5 dithiobiurea and ethylene glycol (NCI, 1979; NTP, 1993). Similarly, hyaline degeneration is not a selective pathological indicator for the early stages of carcinogenesis now and would not have been so in the 1930s-60s. Historically, it was identified as a marker of "disturbances of liver function" in 1938 (Smith and Gault, 1938). A 1959 textbook indicated "hyaline droplets" were observed in hepatic cells in certain cases of cirrhosis associated with alcoholism, "masses of hyaline material" in the

¹⁷ The 3rd (1938) and 4th (1943) editions of Boyd's pathology textbooks both stated that mitotic figures may be seen while the 6th (1953) edition stated mitotic figures were "suggestive."

cytoplasm of cells with yellow fever, or “hyaline bodies” in the cytoplasm of necrotic liver cells in acute hepatitis (Smith, 1959). The term “Mallory bodies” was recognized as a change that occurred as a result of alcoholic hepatitis, long-term experimental administration of griseofulvin and phalloidin, long-standing cholestasis, nonalcoholic cirrhosis, Wilson’s disease, and Indian childhood cirrhosis (King *et al.*, 1983). Thus, hyaline bodies are not specific to cancer and are not markers for the early stages of carcinogenesis, and have never been recognized as such. DeGrandchamp admitted this in an earlier deposition, when he acknowledged that hyaline bodies can be observed in fibrosis of the liver, hepatitis, wound healing, and the normal phenomenon of aging (DeGrandchamp, 2018).

3. Dr. DeGrandchamp’s assertions that bioaccumulation and biomagnification were scientifically defined concepts by 1945 are incorrect and do not reflect the state of scientific knowledge during this time period

Dr. DeGrandchamp’s statements that “The scientific industry understood principles of bioaccumulation well before Monsanto began producing PCBs,” “Monsanto must have known by 1945-1950 that PCBs bioaccumulate and biomagnify if released into the environment,” and “As early as 1945, and by no later than 1950, Monsanto must have known that PCBs would bioaccumulate and biomagnify in humans and animals” (DeGrandchamp Expert Report, Oct. 11, 2019, summary of opinions, p. 97, 138-139) are incorrect and do not represent the state of scientific knowledge during this time period. Rather Dr. DeGrandchamp’s statements reflect his own scientific knowledge based on his current understanding of today’s literature. As detailed below, Dr. DeGrandchamp’s statements are incorrect and do not reflect the state of scientific knowledge during this time period regarding bioaccumulation. Monsanto could not have known PCBs would bioaccumulate and biomagnify by 1950 because, as detailed below, these scientific concepts would not be established until the mid- to late-1950s and early 1960s.

Dr. DeGrandchamp defined bioaccumulation as “a gradual increase in PCB body burden that results from the net between absorption of PCB into the body minus its elimination from the body. When rate of intake and absorption of PCBs into the body exceeds the rate of excretion from the body, PCBs bioaccumulate with continued exposure. The body burden is the net sum of PCBs measured in the body at a particular point in time” (no citation; DeGrandchamp Expert Report, Oct. 11, 2019, p. 97) and biomagnification as “the accumulation of organic compounds (e.g., PCB and DDT) by animals and humans from chemical intake that results in a body burden that is greater than the intake concentration. This describes the increase or magnification of the body burden at each trophic level moving up the food chain. Because humans sit at the apex of the food chain, the body burden will be highest in man” (no citation; DeGrandchamp Expert Report, Oct. 11, 2019, p. 99). These definitions are consistent with contemporary definitions of these terms (Hayes, 2014).

Dr. DeGrandchamp failed to understand that generally-recognized scientific principles known today were not generally accepted (or known) during the 1930s, 1940s, and 1950s. His analysis misrepresented scientific knowledge at the time regarding the concepts of bioaccumulation and biomagnification.

Bioaccumulation: Although bioaccumulation is a prominent term in contemporary health literature, it was non-existent prior to the 1960s. A search of the PubMed database failed to find a single article using key

words beginning with “bioaccumulat___”, “biomagnif___” or “bioconcentrat___” through the 1950s.¹⁸ In order to illustrate the timeline of publications (and thereby the state of scientific knowledge regarding bioaccumulation, biomagnification and bioconcentration), I generated a graph for the number of articles published in Pubmed containing words beginning with “bioaccumulat,” “biomagnif” or “bioconcentrat” (generated using a wild character in the search string) across decades of interest through the present;¹⁹ see Figure 17.

I also searched the American Chemistry Society (ACS) Publications²⁰ and GoogleScholar²¹ for publications with these same words through the 1960s. No citations were retrieved from ACS and two applicable citations was retrieved using GoogleScholar. A 1957 citation associated with the term “bioconcentration” related to mercury and methylmercury in marine fish (Corner and Sparrow, 1957). The other identified citation mentioned the term “bioaccumulation” related to radioisotope research in 1958 in a study reporting the amount of bioaccumulation by aquatic organisms varied over many orders of magnitude depending upon the kinds of isotopes involved and many physical, chemical, and biological factors (Davis and Foster, 1958). Key processes affecting bioaccumulation of radioactive materials included (1) Absorption, which was a function of exposed areas, tissue absorption characteristics, and assimilation of absorbed/ingested material; (2) Retention, which was a function of deposition site, turnover rate, and radioactive half-life; and (3) Elimination modes, which included ion exchange, diffusion, excretion, and defecation (Davis and Foster, 1958).

¹⁸ PubMed comprises over 28 million citations for biomedical literature from MEDLINE, life science journals, and online books (<https://www.ncbi.nlm.nih.gov/books/NBK3827/#pubmedhelp.FAQs>).

¹⁹ National Center for Biotechnology Information (NCBI). PubMed database. Searched April 24, 2019 using individual search strings for bioaccumulat*, biomagnif* and bioconcentrat*. <https://www.ncbi.nlm.nih.gov/pubmed>.

²⁰ Google Scholar. <https://scholar.google.com>. Searched April 24, 2019 using bioaccumulation, bioconcentration, or bioaccumulation.

²¹ American Chemistry Society (ACS) <https://pubs.acs.org>. Searched April 24, 2019 using bioaccumulat* or biomagnif* or bioconcentrat.

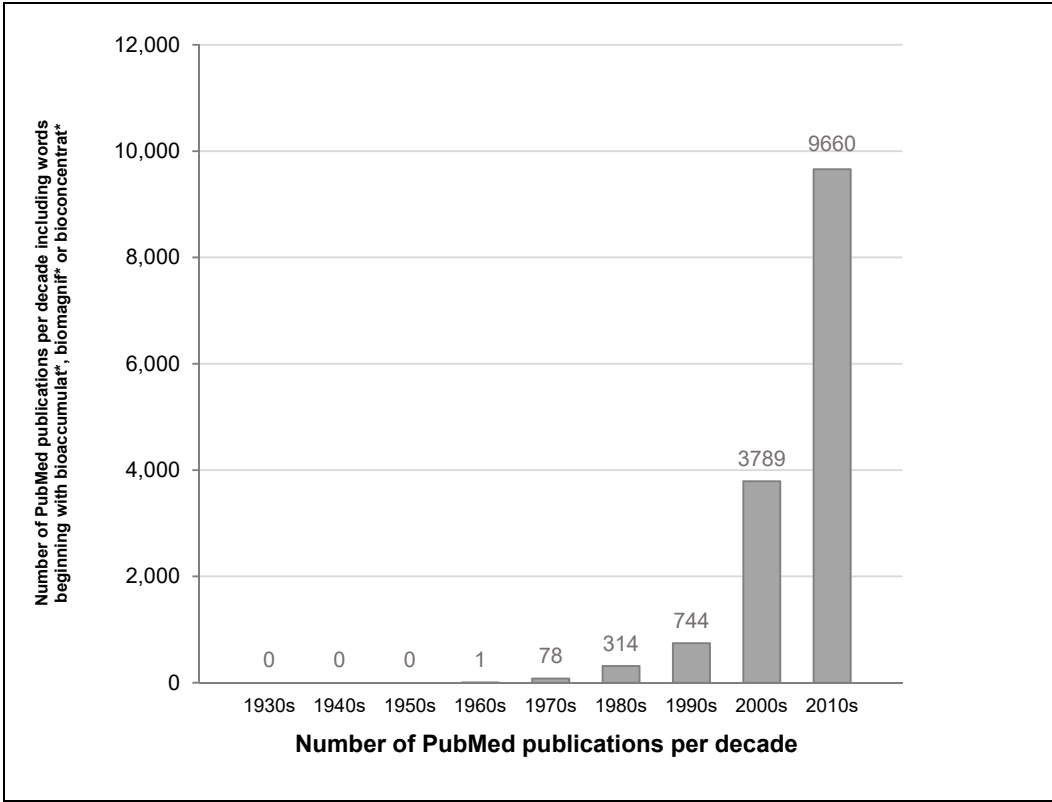


Figure 17. PubMed Publication Timeline.
Counts represent number of publications retrieved in PubMed searches generated using the search strings bioaccumulat*, biomagnif* or bioconcentrat*. Numbers above bars indicate number of publications in the applicable decade.

The essential absence of the term, “bioaccumulation” from the scientific literature prior to the 1970s likely reflected the non-existent or early stages of the evolution of the concept. In the 1940s and 1950s, there were some reports that had detected DDT concentrations in fat stores (and milk secretions) among animals that had been fed DDT-containing feed. Although the observation met the contemporary definition of bioaccumulation, science had not yet coined the definition for the concept and the studies represent the forefront of science at the time. These studies would lay the foundation for later observations, hypotheses, and eventual experimentation leading to the discovery and characterization of the processes of biomagnification. In particular, bioaccumulation would not be conceptualized until the parameters of bioconcentration and biomagnification by aquatic organisms were recognized. With this recognition in the mid- to late-1950s and early 1960s, levels of bioaccumulation potentially possible via food chain bioaccumulation far surpassed those assumed to occur from ingestion of treated or contaminated foodstuffs alone. Only then did the potential for bioaccumulation of chemicals not directly or indirectly added to foods become a potential human health concern.

The literature was evaluated for the existence of when key events, observational studies, and/or hypotheses that led to the discovery that food chain biomagnification occurred. Studies that first hinted the process could occur were not present until the mid-1940s (e.g., radiation fallout from World War II and test

detonations enabled use of highly specific and sensitive radionuclides to explore pathways of air and water transport, as well as the pathways along which pollutants were distributed in plant and animal communities (Woodwell, 1967). In the mid- to late-1950s, when researching the cause of a central nervous system disease affecting people (and developing babies) living near Minamata Bay in Japan (e.g., “Minamata Disease”), researchers published the first instance whereby an environmental pollutant had accumulated through the food chain (i.e., mercury discharged into the Bay had accumulated as through the aquatic food chain as methyl mercury, which was consumed in fish) (Hayes, 2014). Around that same time the first studies were published that found/suggested unexpectedly high concentrations of insecticides used to treat pests that adversely affect food crops and/or transmit human disease (e.g., DDT and its analog DDD) in wildlife organisms. Specifically a 1958 study (Barker, 1958) reported robin deaths near DDT-sprayed elm trees; study authors implicated earthworms had concentrated DDT to levels that proved lethal to birds who consumed them. A 1960 study (Hunt and Bischoff, 1960) reported DDD, an analog of DDT that had been across multiple years to Clear Lake (California) as an insecticide, observed bioaccumulation of the insecticide up the food chain (i.e., it accumulated up the trophic levels in the lake habitat).

To summarize, Dr. DeGrandchamp’s speculation that “The scientific industry understood principles of bioaccumulation well before Monsanto began producing PCBs,” “Monsanto must have known by 1945-1950 that PCBs bioaccumulate and biomagnify released into the environment,” and “As early as 1945, and by no later than 1950, Monsanto must have known that PCBs would bioaccumulate and biomagnify in humans and animals” is scientifically invalid because it does not accurately reflect historical scientific knowledge of bioaccumulation and biomagnification during the time period. Monsanto could not have known PCBs would bioaccumulate and biomagnify by 1950 because at that time, these scientific concepts were unknown to the scientific community.

4. Dr. DeGrandchamp’s incorrectly used the term “biomagnification” and made numerous factual mistakes in his interpretation of the studies upon which he relies

As stated above, extrapolation of the concept of biomagnification to periods preceding the mid-1950s is scientifically invalid since the concept had not yet been identified, defined or researched. Dr. DeGrandchamp failed to recognize this and repeatedly applied the term before the concept was scientifically known. Furthermore, as specified below, Dr. DeGrandchamp’s use of the term contradicts his own definition and the contemporary definition of biomagnification.

Dr. DeGrandchamp defined biomagnification as “the accumulation of organic compounds (e.g., PCB and DDT) by animals and humans from chemical intake that results in a body burden that is greater than the intake concentration. This describes the increase or magnification of the body burden at each trophic level moving up the food chain. Because humans sit at the apex of the food chain, the body burden will be highest in man” (no citation; DeGrandchamp Expert Report, Book 2, October 11, 2019, p. 99). This definition is consistent with the contemporary definition for biomagnification - whereby contaminant concentrations of tissues in higher trophic levels are successively higher due to dietary accumulation (e.g., benthic organisms have the lowest tissue contaminant concentrations; small aquatic organisms that eat those benthic organisms accumulate higher levels in their tissues than the benthic organisms; small fish that eat those small aquatic organisms accumulate higher tissue levels; and so-on such that the tissue concentrations are highest (and are magnified) in the longest-living/largest organisms – such as whales) (Hayes, 2014). Examples where

Dr. DeGrandchamp misinterprets study results and/or misuses the concept of biomagnification are listed below.

Telford and Guthrie (1945)

Dr. DeGrandchamp incorrectly specified that “Telford and Guthrie also showed that the DDT can be continuously transported through the food chain, and their findings showed biomagnification” (DeGrandchamp Expert Report, Oct.11, 2019, p. 152). A review of the Telford and Guthrie (1945) study shows the purpose of the study was to determine if DDT was eliminated through the milk of rats or goats fed relatively high doses of DDT. The presence of DDT (or an active DDT residue) was determined through observations of animals consuming milk from DDT-treated animals. Recalling that biomagnification involves increasing body burden with increasing trophic levels, this study design provided no opportunity to assess biomagnification. The correct ad hoc assignment of today’s known scientific concepts to this historical study would be bioaccumulation - not biomagnification. Neither concept was scientifically established at the time of the study, which may have contributed to Dr. DeGrandchamp’s error.

Fitzhugh (1948)

Dr. DeGrandchamp incorrectly discusses results of the Fitzhugh (1948) study in the context of biomagnification (DeGrandchamp Expert Report, Oct. 11 2019, p. 156). The Fitzhugh study evaluated levels of DDT in fat of rats fed dietary levels (10 to 600 ppm) of DDT for 2 years, which corresponds to a bioaccumulation study. There was no evaluation of DDT levels between trophic levels, thus characterization of study results as a biomagnification study was scientifically incorrect.

Dr. DeGrandchamp continues to incorrectly use the term biomagnification in the discussion of the Fitzhugh (1948) study (DeGrandchamp Expert Report, Oct. 11, 2019, p. 158). Specifically, Dr. DeGrandChamp opines that “[s]ome of the rats had a biomagnification factor of up to 27-fold.” Again, although neither concept was scientifically established at the time of the study, the correct ad hoc assignment would be bioaccumulation - not biomagnification.

The discussion of the Fitzhugh (1948) study by Dr. DeGrandchamp incorrectly concluded that “[t]he 1948 Fitzhugh study presented clear, simple, and unequivocal empirical evidence that DDT bioaccumulates and biomagnifies” (DeGrandchamp Expert Report, Oct. 11, 2019, p. 159). Dr. DeGranchamp is opining that the Fitzhugh study demonstrates both bioaccumulation and biomagnification. However, as specified above, the study did not evaluate concentrations between trophic levels and thus characterization of this study as a biomagnification study is scientifically incorrect.

5. Dr. DeGrandchamp misrepresents conclusions of the studies he cites

Much of Dr. DeGranchamp’s discussion in support of his opinion that “*Monsanto must have known by 1945-1950 that PCBs bioaccumulate and biomagnify*” is speculative, uncited, or relies on citations from 1970 or later²² (DeGrandchamp Expert Report, Oct. 11, 2019, pp. 139-143). When Dr. DeGrandchamp included citations from the relevant time period (i.e., 1940s to 1950s) to support his opinion, he misrepresented the

²² DeGrandchamp Expert Report, Oct. 11, 2019, pp. 139-143, includes three citations [91-93] dated 1975, [undated citation for US EPA; same title is available as a web page whose last update was dated August 11, 2017, <https://www.epa.gov/ingredients-used-pesticide-products/ddt-brief-history-and-status>], and 2019.

conclusions of the authors. Examples of Dr. DeGrandchamp's misrepresentation of study authors' conclusions are included below.

Woodard et al. (1945)

Dr. DeGrandchamp discusses the Woodard *et al.* (1945) study in his expert report on pages 149-151 where he misrepresents the authors' conclusions regarding DDT. Dr. DeGrandchamp failed to provide an accurate summary of the study, which was a preliminary evaluation of fat sample analyses from only 4 male and 4 female dogs whose exposures consisted of 10, 50 or 80 mg/kg for 138, 443, or 747 days. DDT was detected in milk samples from a single dog fed DDT at 80 mg/kg/day. Milk from another dog, fed an isomer of DDT, was found to contain the isomer of DDT. Thus, it was a small study that detected DDT in body fat of dogs given large (at least 10 mg/kg) daily doses and in the milk of a dog fed 80 mg/kg/day. In his summary of the article, Dr. DeGrandchamp stated Dr. Woodward and his colleagues "*theorized that the lipid solubility of DDT enables it to be absorbed into the female body, where it bioaccumulates in the fat-rich breast tissue during pregnancy and that stored DDT is secreted into breast milk. The DDT in breastmilk would then be absorbed by the suckling offspring to bioaccumulate in the bodies of offspring. This study showed not only that DDT could be transported through livestock and food chains to ultimately target human newborns, but that absorption by livestock could be very significant*" [citation to Woodward *et al.*, 1945] (DeGrandchamp Expert Report, Oct. 11, 2019, p. 149).

Many aspects of the Woodard *et al.* (1945) publication were misrepresented by Dr. DeGrandchamp. Contrary to what Dr. DeGrandchamp presented, the Woodard *et al.* (1945) publication did not include any discussion of accumulation in fat-rich breast tissue, absorption by offspring, accumulation in bodies of offspring, targeting of human newborns, transport through livestock, transport through food chains, or any discussion of absorption by livestock.

Telford and Guthrie (1945)

On page 152 of his expert report, Dr. DeGrandchamp includes an apparent excerpt [quotation] from a study by Telford and Guthrie (1945); a portion of the applicable page from Dr. DeGrandchamp's expert report is included below. Notably, only one of the italicized sentences (the first one) is from the Telford and Guthrie publication. The second sentence is apparently composed by Dr. DeGrandchamp (for which he transposes current scientific knowledge into results of historical studies); Dr. DeGrandchamp misrepresented the second sentence that was written in 1945 by Telford and Guthrie. Fabrication of an authors' conclusions is not a generally accepted practice in the scientific community.

died within 2–9 days; milk from goats fed DDT for longer periods was more toxic. Telford and Guthrie stated: <i>Milk obtained from goats having received these dosages from 21 to 26 days was much more toxic than milk obtained from animals subjected to shorter periods of treatment.</i>	Accurate quotation from Telford and Guthrie, 1945
<i>This indicated that DDT continued to bioaccumulate with continued exposures and once in fat tissue it remained for significant periods of time and was not rapidly eliminated.</i>	Not part of Telford and Guthrie, 1945.

Excerpt from DeGrandchamp Expert Report, Oct. 11, 2019, p. 152.

Bishopp (1946)

Dr. DeGrandchamp’s presentation of the June 1946 editorial in the American Journal of Public Health (Bishopp, 1946), which he stated was a position paper by the American Public Health Association (APHA) that “was essentially a cautionary statement noting that ... exposures to humans was a real concern and must be taken into account.” In actuality, the publication was a review of DDT for insect control that also included human toxicity. Dr. DeGrandchamp failed to present the authors’ conclusions regarding the toxicity to humans: “*When used as recommended for the control of human parasites and household insects, DDT insecticides are not harmful to human health. This includes DDT in aerosols and the several other forms in which it is recommended*” and “*If the formulations and time and method of application are carefully chosen, minimum dosages and number of applications are used, and the material is employed only when needed to combat a pest for which it is known to be an effective remedy, it is confidently believed that no serious consequences will result from the use of DDT*” (Bishopp, 1946). When the totality of Bishopp’s conclusions presented in his 1946 paper are considered, it is clear Dr. DeGranchamp mischaracterized the paper with his synopsis that the paper “*was essentially a cautionary statement noting that ... exposures to humans was a real concern and must be taken into account. APHA believed it was its professional responsibility to issue a caution that DDT is highly lipophilic and will bioaccumulate and biomagnify if used to spray crops or directly applied to human skin in an oil-DDT formulation because no regulations existed in 1946 regarding the judicious and safe use of DDT*” (DeGrandchamp Expert Report, Oct. 11, 2019, p. 153). **This is a complete misrepresentation of the APHA report, as shown above.**

6. Dr. DeGrandchamp’s analysis regarding the historical knowledge of DDT bioaccumulation and biomagnification was scientifically flawed

Misinterpretation of the historical scientific studies of DDT

As discussed in previous sections, Dr. DeGrandchamp’s analysis regarding the historical interpretation of DDT studies was scientifically flawed because he misinterpreted the historical scientific studies of DDT. Dr. DeGrandchamp presented historical studies but inserted his own commentary regarding conclusions for concepts that were not established until decades later. In so doing (as described above), Dr. DeGrandchamp often over-interpreted results and presented his own interpretation as if it was the authors’ original conclusion(s).

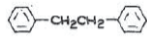

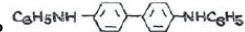
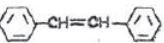
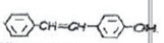
Inference that PCBs would behave like DDT, based on the 1945-50 studies on DDT, is not supported by the science from that time period

Although many of the studies were misinterpreted by Dr. DeGrandchamp, the series of studies he presented generally reflected the historical evolution of DDT research. Importantly, the early studies from the mid- to late-1940s were the beginning of the scientific investigation regarding the accumulation of DDT in body fat. Studies considered by Dr. DeGrandchamp to be “salient” research for bioaccumulation and biomagnification [of DDT] during 1945-1950 (DeGrandchamp Expert Report, Oct. 11, 2019, p. 139) were actually very early scientific studies of DDT and cannot be used to infer all lipophilic compounds would be expected to act in a similar fashion. Thus, inferring that PCBs would behave like DDT, based on the 1945-50 studies on DDT, is not supported by the science from that time period

The potential for human toxicity of DDT was of obvious interest given its prevalent use and human exposure (e.g., on food crops, on humans, and in residences). DDT was designed as a biological poison intended to kill living organisms (e.g., insects), and was dispersed in huge quantities directly into the environment, and directly onto people. Thus, the relevance of comparison of DDT to PCBs is questionable, at best. In 1945, Woodard *et al.* hypothesized the high lipid-water distribution ratio of DDT may suggest a propensity for preferential adipose tissue storage in mammals fed diets containing DDT; their study results supported their hypothesis. A few studies followed in the 1940s to attempt to characterize the propensity for DDT to accumulate in the fat of animals who were fed large quantities. It was not until the mid- to late-1950s/early 1960s that scientific knowledge would begin to expand and reveal that biomagnification of environmental pollutants could occur (Barker, 1958; Hunt and Bischoff, 1960). It was longer still before scientific publications documented a stepwise increase in DDT concentration from one trophic level to the next, suggesting a food-chain transfer (Hamelink *et al.*, 1971; Woodwell *et al.*, 1967). Based on the timeline of scientific knowledge, Dr. DeGrandchamp’s assertion (p. 139) that “*Given the similarities between DDT and PCBs, the industry-wide knowledge of DDT bioaccumulating and biomagnifying, and the fact that Monsanto manufactured DDT, Monsanto must have known by 1945-1950 that PCBs would bioaccumulate and biomagnify if released into the environment*” is **inconsistent** with historical studies.

7. DeGrandchamp incorrectly states that Monsanto should have known that PCBs might be carcinogenic because of their chemical structure

In spite of Dr. DeGrandchamp’s suggestion that PCBs are similar to PAHs, from a chemistry perspective there is nothing remotely similar between the structure of PCBs and the structure of PAHs, because the primary chemical characteristic common to PAHs is condensed benzene rings (the rings share common carbon atoms). While PCBs have two benzene rings, they are not condensed, and thus do not have the ‘aromaticity’ that is essential for the carcinogenic action of some of the hundreds of PAH molecules found in coal tar, soots and other sources of combustion of organic materials (in fact, the large majority of PAH molecules are not carcinogenic, because of subtle structural differences that modify how easily a particular carbon in the aromatic ring structure can be oxidized). The same can be said for azo dyes, aromatic amines, heterocyclic amines, and steroid molecules, all of which make up 90+% of the studies in the Hartwell compendium that were listed as ‘positive’ (the remaining 10% were studies on metals such as arsenic, lead and nickel, and endogenous steroids such as estrogen and cholesterol).

Dr. DeGrandchamp further suggests that PCBs and benzidine are 'similar', and since benzidine is carcinogenic, scientists should have presumed that PCBs might be carcinogenic (DeGrandchamp report, Book 1, p. 14-15; Figure 1). What he fails to mention is that the Hartwell report shows NO indication that benzidine was carcinogenic at that time. Chemical 628 (Benzidine) in the Hartwell compendium shows 8 different studies, of which only 1 showed any evidence of tumors, and that was when benzidine was painted onto the skin. It wasn't until 1950 that animal studies showed that benzidine could cause tumors when injected subcutaneously into rats (Spitz *et al.*, 1950). Indeed, using his logic, a review of the compounds that are in the Hartwell report that are somewhat structurally similar to PCBs would have strongly argued that they would NOT likely be carcinogenic. For example, 'dibenzyl' (compound 639 ) , diphenyl itself (compound 652; ) , diphenylbenzidine (compound 653 ) , stilbene (compound 677; ) , and 4-hydroxy stilbene (compound 663; ) were listed in the compendium, with no evidence of tumorigenicity for ANY of them. Thus, in complete contrast to Dr. DeGrandchamp's supposition that the chemical structure of PCBs should have been a 'flag' that it might be carcinogenic, all of the evidence available at that time, including the evidence compiled in the Hartwell compendium, would have argued exactly the opposite- the studies available on structurally similar compounds provide NO evidence that PCBs might be carcinogenic.

Degradchamp also states "As previously discussed, the benzenes that Monsanto used in the first step of making PCBs were actually derived from coal tar. Since coal tars had been known to be carcinogenic, this alone should have been a trigger for testing. However, another trigger that was identified at the time was the isolation of pure biphenyls from coal tar. That is, by 1927, biphenyls were shown to be part of the coal tar brew of chemicals. Therefore, it was known by 1927 that coal tar caused cancer and that both benzene and biphenyls were chemicals found in coal tar. This, by itself, should have constituted a trigger since PCBs are biphenyl compounds." (Degrandchamp Book 1, p. 15-16). While biphenyls and many other organic molecules are indeed present in coal tar, it was widely recognized in the 1930s and on that it was the polyaromatic hydrocarbons (PAHs) that were largely, if not solely, responsible for the carcinogenicity of coal tar mixtures. Indeed, the vast majority of compounds studied in the first Hartwell compendium were PAHs, with the goals of understanding exactly which PAH molecules were carcinogenic, and which were not. To suggest that it would be obvious that PCBs were carcinogenic in the 1930s just because biphenyl is present in coal tar is a ridiculous statement with no foundation in science, given the knowledge of the time. As noted above, benzidine was not recognized as a carcinogen until the 1950s. Although occupational exposure to benzene had been linked to leukemia in the late 1920s, it wasn't until 1979 that benzene was shown to cause cancer in experimental animals (Huff, 2007), further demonstrating that an attempt at an animal study to evaluate the potential carcinogenicity of PCBs in the 1930s, 40s or 50s would not have been successful.

Further, the presence of multiple chlorine atoms on the two benzene rings completely change the nature of these molecules, because the chlorine atoms protect the carbons in the aromatic rings from oxidation. This is in fact what made these molecules so useful in their day. They are remarkably heat stable, and are non-flammable and non-explosive, explicitly because the chlorines on the two rings protect the carbons from oxidation. This was well understood in the 1930s, and, is why no reasonable scientist familiar with the

chemistry of known carcinogens in those days (e.g., PAHs, azo compounds, and aromatic amines) would ever conclude that PCBs would be reasonable candidates for carcinogenicity testing.

Thus, it is my opinion that there was no reason to suspect that PCBs might be carcinogenic, and numerous reasons to think they probably would not be carcinogenic, based on the knowledge of chemical carcinogenesis in the 1930s, 40s and 50s.

8. Comments on Degrandchamp opinions expressed in 'Book 3' dated Oct. 11, 2019:

Degrandchamp Opinion 2: "The current fish advisories issued by the WDOH are scientifically tenable and are necessary to protect Washington state residents."

Comment: The "current" fish advisories issued by the WDOH utilize toxicity values (e.g., cancer slope factors, Reference Doses) that are based on EPA's 20+ year old values. There are two major assumptions that go into the EPA/WDOH cancer risk assessment (which may have been reasonable default assumptions in 1996) for which current science in the past 20 years demonstrate to be completely wrong: 1) The EPA/WDOH risk assessment for cancer assumes a linear response at low doses (non-threshold type of cancer risk). As discussed in length in my report, there is now overwhelming evidence to demonstrate that the liver tumors upon which the EPA's cancer 'slope factor' of 2.0 per mg/kg/d was determined are the result of molecular events that require the activation of the aryl hydrocarbon receptor (AhR). In the absence of significant and prolonged activation of the AhR, there is NO cancer risk, because the critical molecular event simply does not happen. Thus, there IS a 'threshold' at which the cancer risk drops to zero – the maximum dose, in humans, that has no measurable effect on the human AhR and other nuclear receptors such as CAR and PXR. Thus, the shape of the dose-response curve at low, 'environmental' doses of dietary PCBs follows a threshold, rather than linear, response, and the current levels of exposure from PCBs in Spokane River do not exceed this threshold. **In fact, these levels are tens of thousands of times below the threshold for significant activation of the human AhR, as demonstrated in numerous studies that have looked directly at this question.** 2) The EPA/WDOH risk assessment uses 'toxic equivalence factors' (TEFs) to calculate the theoretical cancer risk for dioxin-like PCBs. But they rely upon WHO 2005 TEF values, which have been shown in the past 5-10 years to be highly inaccurate for humans. Recent studies have demonstrated that only 1 of the 12 'dioxin-like PCBs' determined in rats is active toward the human AhR: PCB126. Thus, inclusion of PCB118 and other DL-PCBs_(rat) in TEQ calculations for the human risk assessment is inappropriate. Further, the TEF used for PCB126 was the old value of 0.1, set more than 20 years ago, based on comparative potencies of different DL-compounds toward activation of rat AhR. Recent studies, particularly Larsson *et al.* (2015), have unequivocally demonstrated that the response of the human AhR to PCB126 is much less than toward rat AhR, and human tissue-based studies derive a TEF of 0.003, rather than 0.1, demonstrating that PCB126 is over 300 times LESS potent toward activation of the human AhR, compared to dioxin (TCDD) in the human AhR. Further, TCDD is about 26 times less potent toward the human AhR when compared to the rat AhR, making the species difference in susceptibility to the carcinogenic effects of PCBs even greater. In the absence of ANY measurable activity of DL-PCBs other than PCB126, it becomes evident that the EPA/WDOH cancer risk assessment for PCBs in fish is completely wrong. Even if one were to (incorrectly) assume a linear, low dose, response for PCB and cancer, their risk estimation overestimates risk by several thousand-fold, putting the theoretical risk well within the '*de minimus*' risk levels of 1×10^{-5} used by WDOH.

WDOH/ATSDR chose to use the EPA 1996 cancer potency factor of 2.0 per mg/kg/d, based on 2-year cancer bioassays in rats. In fact, EPA calculated a whole range of cancer potency factors from a variety of different studies (1996 EPA cancer potency estimates provided earlier in my report as Table 6, p. 35).

It is evident in looking at data in Table 6 that a slope factor of 2 represent a highly conservative (likely to over-estimate risk) value when looking across the various studies. It is based largely on the response of female rats to Aroclor 1260. Yet the profile of PCB congeners found in fish is much more similar to Aroclor 1242 and 1248, than to Aroclor 1260 (Kostyniak *et al.*, 2005). The EPA potency factor calculated for Aroclor 1242 (0.4 for female SD rats) is 5 times less than the value (2.0) selected for use by the EPA. And, as noted numerous times previously, the studies on TCDD and specific PCB congeners (118, 126 and 153) conducted in 2004-2006 by the National Institute of Environmental Health Sciences (NIEHS) clearly delineate the mode of action as requiring significant and prolonged activation of the AhR, with a steep dose-response and clear threshold levels (NTP, 2006a, c, d). Thus, using a slope factor for a linear response at low doses does not represent the current state of knowledge of the carcinogenic properties of PCBs found in fish. The most scientifically valid approach, using all of the current knowledge we have on the mode of action of PCBs in causing cancer in laboratory rats and mice, is to use an 'Adverse Outcomes Pathway' approach to: 1) identify a 'key molecular event' required for carcinogenic response (which is activation of the AhR), 2) determine a reasonable but conservative 'Point of Departure (POD)', which is the dose (in mg/kg-d) necessary to initiate the key molecular event, 3) use current scientific information to adjust for known species differences to calculate a human-relevant POD, 4) estimate the human dose from consumption of relevant PCB congeners in fish and 5) calculate 'Margins of Exposure' to determine how close the dose from consumption of fish might be to the 'target dose' (human-based Point of Departure) necessary to trigger the key molecular events necessary for cancer to develop. **I have done that in my report, and it demonstrates that Margins of Exposure, for even the extremely high, hypothetical/impossible scenario (upper 95% percentile of fish consumption rates times 95% of relevant PCB congener concentrations), far exceed 100,000 for cancer, immune toxicity, liver toxicity and reproductive effects that are largely if not exclusively mediated via activation of the AhR.**

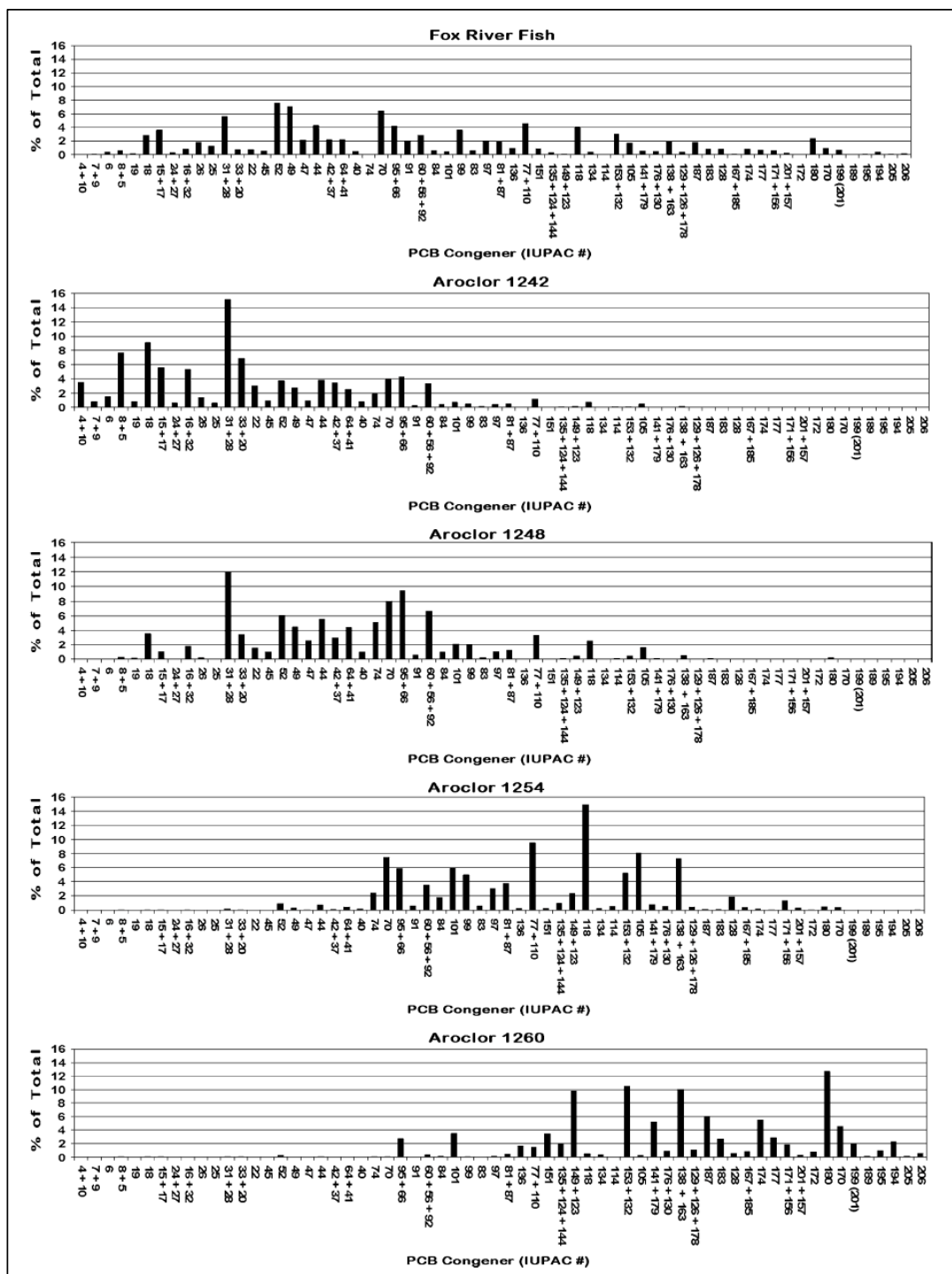


Figure 18. Fish and Aroclor congener profiles (Kostyniak *et al.*, 2005)

Degradchamp Opinion 3: “The ATSDR/WDOH Health Consultation’s recommendations, if followed, will reduce the health threat posed by eating PCB-contaminated fish while maximizing the greatest number of fish meals that can be consumed without fear of PCB-induced toxic effects.”

Comment: As noted above, there is NO PCB-related cancer risk from consuming Spokane River fish because the doses received, even when added to background, are hundreds of thousands of times below those necessary to trigger biological events that could potentially lead to cancer, immune disruption and reproductive effects. Theoretical neurodevelopmental effects from PCBs would likely become the ‘driver’ for clean-up levels if EPA and WDOH were to use current science in their risk assessment. But even for this theoretical endpoint, the science is weak, and the shape of the dose-response relationship is such that thresholds are again very likely to be present. My best estimation of ‘Margins of Exposure’ for this endpoint still demonstrated exposure several orders of magnitude below a level of concern. Because of the presence of other toxic substances, such as PBDEs, lead and mercury, it is likely that fish advisories would still exist, even if they were not based on PCB consumption. Dr. Degrandchamp’s statement that fish could be “consumed without fear of PCB-induced toxic effects” is rather ironic, given that the perception of such ‘fear of toxic effects’ is largely being driven by outdated science used by WDOH and the EPA, and Dr. Degrandchamp’s own incorrect, outdated opinions.

Degradchamp opinion #4: “The risk assessment presented in the latest 2011 WDOH Health Consultation shows that PCBs pose a cumulative lifetime cancer risk of 1.1E-4 (one-in-ten-thousand). This risk levels far exceeds the de minimis acceptable risk level by 100-fold and to reach acceptable risk levels the fish tissue levels must be reduced by 100-fold.”

Comment: My rebuttal to this opinion was addressed above in part in my response to Dr. DeGrandchamps’ first opinion. Further, it is an incorrect representation of the WDOH Health Consultation, which, even without adjustment for well-established species differences and assuming low dose linear response, shows cancer risks in the 10^{-5} range (WA Department of Health, 2011). The table below is taken directly from the WDOH Consultation report. In only one of the 9 different assessments was the projected theoretical risk above 1.10^{-4} (one-in-ten-thousand). For total theoretical risk for consumption of fish fillets (by far the most likely scenario) the risk was 6.4×10^{-5} , or 6 in 100,000. That 10,000 people would, on a regular basis over 30 years, consume 30% of their catch as ‘whole fish,’ including suckers²³ which are seldom eaten (See Sundig Expert Report) is completely unrealistic.

²³ The 2011 WDOH/ATSDR Document states the following regarding ‘whole fish’ consumption: “Whole – Total PCB concentration calculated based on consumption of large scale sucker (whole body) (15%) and bridgelip sucker (whole body) (15%) using the maximum value of the mean concentration for each species.”

Table 19. Theoretical cancer risk for recreational anglers associated with exposure to contaminants of concern in Spokane River fish (Table B6 from: WA Department of Health, 2011)

Contaminant		Mean Concentration (ppm)	Scenarios Recreational angler	Cancer slope factor (CSF) (mg/kg/day) ⁻¹	Cancer risk (recreational angler)
Total PCBs	Fillet	0.1617	Child	2.0	1.8 x 10 ⁻⁵
			Older child		1.7 x 10 ⁻⁵
			Adult		2.9 x 10 ⁻⁵
	Total theoretical cancer risk (fillet) †				6.4 x 10 ⁻⁵
	Whole	0.2838	Child		1.4 x 10 ⁻⁵
			Older child		1.3 x 10 ⁻⁵
			Adult		2.2 x 10 ⁻⁵
	Total theoretical cancer risk (whole) †				4.8 x 10 ⁻⁵
	Total theoretical cancer risk (both fillet and whole fish) ^A				1.1 x 10 ⁻⁴

Cancer risks represent cumulative lifetime exposure from childhood to adulthood

† - Exposure duration is based on 5, 10 and 15 years exposures for a child, older child and adult respectively, resulting in a total theoretical cancer risk for both fillet and whole fish of 30 year exposure.

^A - Total theoretical cancer risks are based on consumption of fish for both fillet and whole fish. This is assuming recreational anglers eat fish 70% of the time as fillet and 30% of the time as whole fish.

It is perhaps useful to put this 'theoretical cancer risk' in perspective. All of these estimates in Degrandchamp's opinion regarding a cumulative lifetime cancer risk use 'upper bound' rather than 'mean' or 'best-estimate' values (consumption rates, PCB concentrations in fish, source of fish, cancer potency factor, etc.), so they are designed from the beginning to substantially overestimate 'real' risk (and that is also the purpose of the incorrect 'linear at low dose' assumption, which is not scientifically defensible for PCBs). The hypothetical scenario that Dr. Degrandchamp chooses to illustrate as 'unacceptable risk' (1.1×10^{-4}) says that, IF 10,000 thousand people consumed Spokane River fish on a weekly basis for 30 years (at the defined amount), and ALL of those fish had an average of 162 ppb of total PCBs, AND 30% of the time they consumed whole suckers, there would be LESS THAN 1 additional cancer in the entire 10,000 population caused by that consumption over 70 years. And again, this assumes that risk is linear at low doses, and that humans are at least as sensitive as laboratory rats, neither of which are true. Thus, the 1.1×10^{-4} risk scenario is completely unrealistic. In the absence of any PCB exposures from fish, in that same population of 10,000 people there will be approximately 4,000 cases of cancer, and over 2,500 cancer-related deaths, that will occur over 70 years (American Cancer Society, 2018) from all causes (genetics, lifestyle, random mutagenesis/errors in DNA repair, diet, alcohol, smoking, radiation, etc).

The concentration value used by WDOH/ATSDR in the 2011 report of 0.1617 ppm (162 ppb) is also a substantial over estimation of fish concentrations in edible fish tissues today.

Table 20. PCB concentrations in Spokane River fish (Table 5 from: WA Dept of Ecology, 2011)

Location and Tissue Type	Total PCB Concentrations Measured by:					
	Aroclor Analysis					Congener Analysis
	1993 ^a	1994 ^b	1996 ^c	1999 ^d	2001 ^e	2005 ^f
Rainbow trout - fillet						
State line	--	--	--	106	--	55
Plante Ferry	918	424	799	891	--	153
Above Monroe Dam*	--	145	76	226	--	73
Ninemile	490	371	76	143	--	
Mountain whitefish - fillet						
Above Monroe Dam	--	568	381	339	--	234
Ninemile	522	139	444	632	--	139
Little Spokane	--	222	145	--	--	--
Upper Lake Spokane	--		--	--	73	43
Lower Lake Spokane	780	113	--	--	--	76
Largescale suckers - whole						
State line	--	--	--	120	--	56
Plante Ferry	2,005	531	530	283	--	122
Above Monroe Dam	--	201	116	445	--	1,823
Ninemile	1,210		345	680	--	--
Little Spokane	--	440	366	--	--	--
Upper Lake Spokane	--	--	--	--	265	327
Lower Lake Spokane	410	820	--	--	357	254

--no data
^a Johnson et al., 1994
^b Ecology, 1995
^c Johnson, 1997
^d Johnson, 2000
^e Jack and Roose, 2002
^f Serdar and Johnson, 2006
 *Same reach as Mission Park

As shown in Table 2 in the Exposure Assessment Chapter of my report (p. 25), the average concentration of total PCBs in rainbow trout (24 samples from 2003 and 6 samples from 2012) was 36.4 ppb (ng/g). The concentration in Mountain Whitefish was 118 ppb. But perhaps more importantly, the WDOH/ATSDR report substantially overestimates consumption rates, particularly of suckers (used in their 'whole fish' consumption estimates). According to the detailed fish consumption analysis completed by Dr. Sundig, consumption of suckers rarely occurs, and contributes a very small amount to total consumption. Dr. Sundig's analysis is based on detailed assessments of interviews with recreational users of the Spokane River system. As shown in Table 3 of my report (p. 26), Dr. Sundig's estimate of average daily fish consumption for consumers is only 4.4 gm/day, and for the upper 95th percentile 16.8 grams per day. The WDOH/ATSDR risk assessment used a value of 42 grams per day of fish for recreational anglers, and 17.5 grams per day for the 'general population.' However, it is not clear what the 'general population' refers to, since there are no commercial

fisheries on the Spokane River that could provide fish to local markets. Thus, the risk estimates provided in the WDOH/ATSDR report substantially overestimated actual exposures- at least by 10-fold- and thus substantially overestimated the theoretical risks presumed to be associated with PCBs in Spokane River fish.

Dr. Degrandchamp also incorrectly attributes the WDOH's 'acceptable theoretical cancer risk level' to be '1 in a million' (1×10^{-6}). He is incorrect by 100-fold. On p. 18 of the WDOH/ATSDR 2011 (WA Department of Health, 2011) document it states the following:

"[W]DOH's approach is to select a risk of 1 excess cancer in 10,000 people exposed (1×10^{-4}) as the level of concern for cumulative cancer risk."

Even the extreme 'worst case' exposure and concentration scenarios used by WDOH/ATSDR (Table 20 above) do not exceed this value, and, as noted above, are a substantial overestimate of theoretical cancer risk even if one assumed that linear extrapolation was scientifically justified (which it is not).

If the Washington Department of Health were to do a 'real world' assessment of the risks of consuming PCBs in fish caught from the Spokane River today, using current knowledge, they would recognize that the actual size of the population 'at risk' (the number of people who regularly consume fish from the Spokane River throughout the year, with 30% of their consumption being whole suckers, year after year, for 30+ years) would be remarkably small, and the likelihood of even a single case of cancer over the next 70 years resulting from such exposures to PCBs is infinitesimally small (for the reasons stated above it is, in all likelihood, zero). However, in this same population of people, approximately 40% of them will develop some form of cancer in their lifetime from all other causes, and about 20% will die from it (American Cancer Society, 2018). After the City of Spokane has spent hundreds of millions of dollars to reduce this theoretical cancer risk, the same number of people in Spokane will die from cancer – it will make NO difference in public health. It is unfortunate that the time, effort and money spent chasing fleetingly small, theoretical cancer risks is wasted in the face of hugely greater cancer risks to the public from smoking, poor diet and nutrition, alcohol abuse, etc.

Degrandchamp opinion #7: "Spokane has taken action to prevent PCB loading to the Spokane River to improve water quality, reduce fish PCB-contaminant levels, lower the body burden levels in people that eat Spokane fish. This effort will have a significant impact on lowering PCB body burden and the risk of PCB-related cancer and non-cancer disease."

Comment: As noted above, plaintiffs' own expert (Dr. Gobus) states that the maximum positive outcome from remediation efforts would be to eventually reduce PCB concentrations in fish by 7.2%. This is of no public health value, since the current levels of PCBs in fish in the Spokane River do not represent any significant risk to public health, even to low-income and/or native populations who may have relatively higher levels of consumption of fish from the Spokane River.

Degrandchamp opinion #9: "From between the 2001-2005 period and 2030, Spokane's remedial activities will have reduced PCB body burdens, cancer risk, and non-cancer hazard quotient by about 11-17%. This

assumes that Spokane performs the future remedial activities discussed in Michael Baker International Experts report.”

Comment: As noted above, this small potential reduction in PCB levels in fish in the Spokane River will have no public health benefit, because the current levels of PCBs in Spokane River fish do not present any significant public health risk to consumers of these fish.

Degradchamp opinion #10: “From between 2012 and 2030, Spokane’s remedial activities will have reduced PCB body burdens, cancer risk and non-cancer hazard quotients by about 5.5-9%. This assumes that Spokane performs the future remedial activities discussed in Michael Baker International’s expert report. This represent a significant reduction in PCB body burden over just an 18-year period and Spokane’s efforts will have the greatest impact on reducing PCB exposures.”

Comment: Dr. Degrandchamp’s cited reductions (from 5.5-9%) overstate the values provided in Plaintiffs’ expert report by Dr. Frank Gobas. In his detailed report, Dr. Gobas states “*Relative to the 2012 time period, these future planned improvements by the City of Spokane are expected to reduce PCB concentrations in fish of the Spokane River by 4.2 to 7.2%, downstream of the City’s discharges*” (Gobas Expert report, p. 15 and 94). Perhaps more illustrative of the remarkably little impact that the proposed cleanup efforts will have is Dr. Gobas’s Figure 24 (reproduced below):

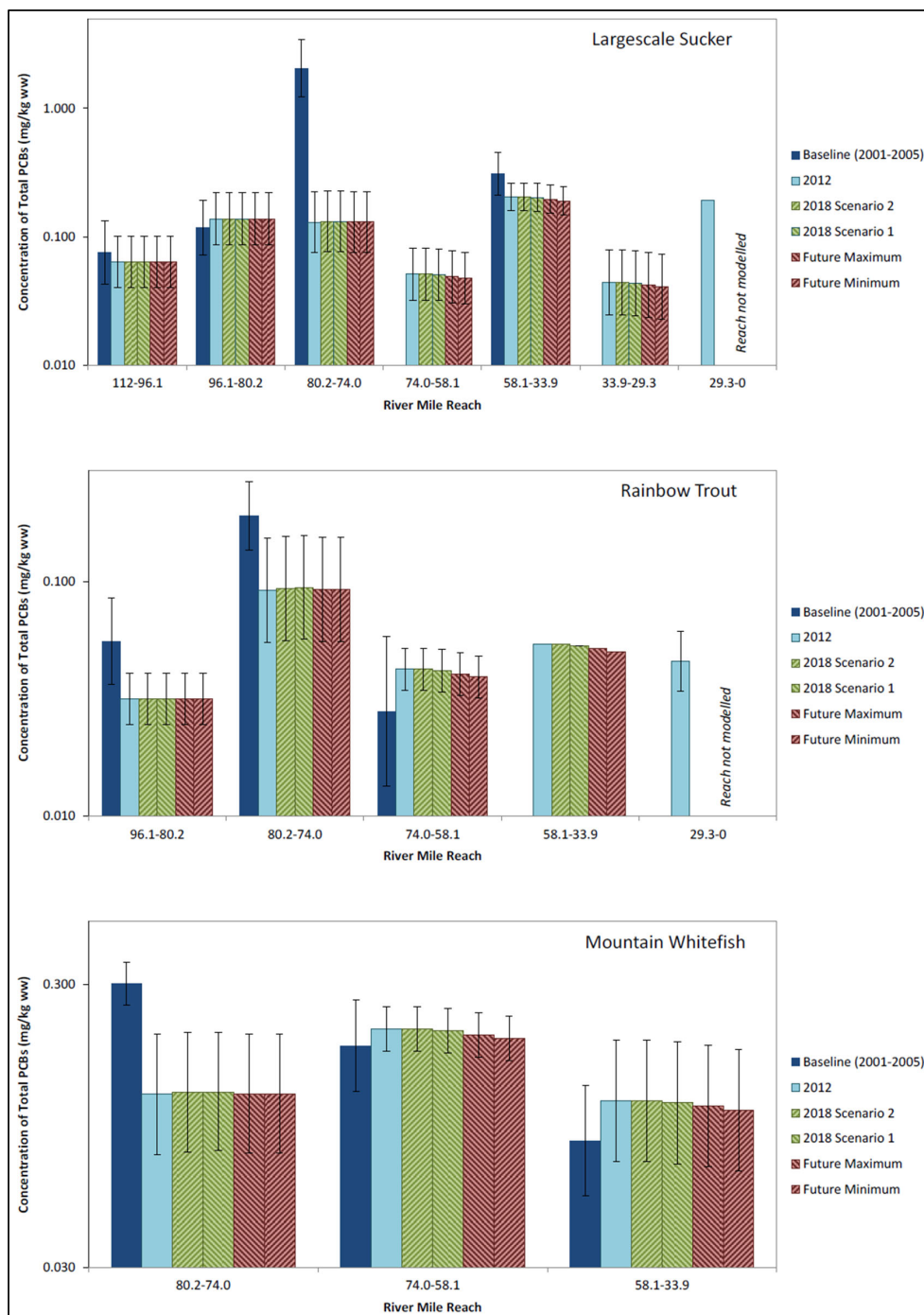


Figure 19. Gobus Report, Figure 24: Baseline, 2012 and predicted current (2018) and future (2030) concentrations of PCBs (mg/kg ww) in fish species in reaches of the Spokane River

It is evident from Figure 19 (reproduced from Figure 24 of the Gobus report) that even the most optimistic prediction (7.2% reduction from 2012 levels over the next 30 years) is completely lost in the noise of the

measurements, as illustrated by the error bars. Although neither Dr. Gobus nor Dr. Degrandchamp note this, it is evident on inspection of the data shown in Figure 19 that there will be NO STATISTICALLY SIGNIFICANT IMPACT on fish PCB levels, even 30 years after remediation. The data in Figure 19 further demonstrate that the WDOH/ATSDR 2011 risk assessment, which used PCB concentrations in fish of 162 ppb, is at least 3-4 times greater than actual levels in Rainbow trout, based on more recent sampling data described in the Exposure Assessment chapter of this report, and in the plaintiffs' expert report by Dr. Gobus.

The WDOH used an estimate of 162 ppb for total PCBs in fish in their risk estimates. Assume that the final maximum reduction in fish tissue PCBs was indeed 7.2% (achieved over many years, not immediately). Thus, the WDOH level of 162 ppb would theoretically go from 162 ppb to 150 ppb (best case; from 162 to 155 ppb if reduction was 4.2%). Or, using the more recent data used in my and Dr. Sundig's expert reports, which is consistent with the 2012 'baseline' values shown in Dr. Gobus's report (see Figure 19 above), levels in rainbow trout might go from 36 ppb to 33 (at 7.2%, or 34, at 4.2%) ppb over the next 20 years, which is completely lost in the analytical 'noise' and variability among fish, and is toxicologically meaningless. Indeed, Dr. Gobus's report demonstrates unequivocally that the \$300M treatment plan will HAVE NO MEANINGFUL IMPACT on the PCB levels in Fish in the Spokane River, even 30 years out. At the miniscule rate of reduction predicted in Dr. Gobus's Plaintiffs' Expert report, the impact on the steady-state levels of blood PCBs (from all sources) in humans would be unmeasurable for decades, because of the long half-life of PCBs in the human body, and because other sources of PCBs that likely contribute more to total PCB exposures will not be reduced by these actions. Thus, the effect on human blood levels of PCBs of consumers of Spokane River fish, if any, would be far less than "5.5-10%" reduction cited by Dr. Degrandchamp. In the end, it would have NO significant impact on blood PCB levels in consumers of Spokane River fish, and no significant impact on their health. It is inconceivable that *"This represents a significant reduction in PCB body burden over just an 18-year period and Spokane's efforts will have the greatest impact on reducing PCB exposures"* (DeGrandchamp Expert Report, Book 3, Oct 11, 2019, p. 3).

Dr. DeGrandchamp misrepresents the ATSDR/WDOH recommendations regarding PCBs. Specifically, Dr. Degrandchamp stated:

"It should be stressed that because the fish advisory is based solely on noncancer health hazards, Washington residents can safely eat the number of fish meals recommended in Exhibit 15. However, if ATSDR/WDOH based their recommendations on de minimis cancer risks from PCBs (as Hg is not a carcinogen), a total ban on eating any fish could be warranted to protect Washington residents from developing cancers" (DeGrandchamp, Book 3, Oct 11, 2019, p. 35).

However, the discussion regarding the potential for cancer in humans from PCBs in fish consumed in the "ATSDR/WDOH" document cited by Dr. DeGrandchamp is much different. Specifically, the report stated:

*The cancer risk associated with chronic exposure at the MRL is estimated at 2 cancers per 100,000 persons exposed over a lifetime. EPA's guidance on fish advisories suggests the use of a target cancer risk of 1 cancer per 100,000 persons exposed **indicating that the noncancer approach is sufficiently protective**" [emphasis added] (WA Department of Health, 2005).*

Furthermore, conclusions from the “ATSDR/WDOH” document, **but NOT mentioned by Dr. DeGrandchamp**, include:

“1. Average consumers of sport fish from Long Lake [of the Spokane River] are not expected to experience adverse health effects from exposure to contaminants in those fish. No apparent public health hazard exists for average consumers of Long Lake sport fish.

2. High-end consumers of sport fish from Long Lake might be exposed at doses only slightly above health comparison values.

- Although these doses are not expected to cause adverse health effects, prudent public health measures such as cooking and cleaning fish to reduce exposure to PCBs through fish consumption can lower potential risk associated with PCBs in fish.” (WA Department of Health, 2005).*

Clearly, the recommendations would not be consistent with a “total ban on eating any fish” as inaccurately presented by Dr. DeGrandchamp.

V. Appendix 1

A. CV of Dr. David L. Eaton

CURRICULUM VITAE

1. BIOGRAPHICAL INFORMATION

DAVID L. EATON, PhD, DABT, FATS

Professor Emeritus of Environmental and Occupational Health Sciences
School of Public Health; University of Washington

Dean and Vice Provost Emeritus, The Graduate School, University of Washington

Adjunct Professor of Pharmacology and Toxicology, College of Pharmacy, University of Arizona

email: toxdok@gmail.com

2. EDUCATION

1978 University of Kansas Medical Center, Kansas City, KS, **PhD**,
(Pharmacology)

1974 Montana State University Bozeman, Montana, **BSc**, (Pre-medicine)

3. LICENSURE / CERTIFICATION

Diplomate of the American Board of Toxicology, 1981, recertified 1985, 1990, 1995, 2000, 2005, 2010, 2015

4. PROFESSIONAL POSITIONS

3/15/2013-3/15/2018 **Dean and Vice Provost**, The Graduate School, University of Washington

10/2010-7/2011 **Interim Vice Provost for Research**, University of Washington, Seattle, WA

2006-2013 **Associate Vice Provost for Research**, University of Washington, Seattle, WA

2003-2019 **Adjunct Professor**, Department of Medicinal Chemistry, School of Pharmacy, University of Washington, Seattle, WA

2000-2019 **Affiliate Member**, Public Health Sciences Division, Fred Hutchinson Cancer Research Center, Seattle, WA

1998-2019 **Professor**, Public Health Genetics Program (UIF), School of Public Health & Community Medicine, University of Washington

1995-2015	Director , Center for Ecogenetics and Environmental Health, University of Washington
1992-2019	Professor , Department of Environmental and Occupational Health Sciences, School of Public Health and Community Medicine, University of Washington
2003-2007	Associate Director , Fred Hutchinson Cancer Research Center / University of Washington / Childrens' Hospital and Medical Center <i>Cancer Center Research Consortium</i> (FHCRC/UW/CHMC CCRC)
1999-2005	Associate Dean for Research , School of Public Health and Community Medicine, University of Washington
1991-1993	Associate Chairman , Department of Environmental Health, School of Public Health and Community Medicine, University of Washington
1986-1991	Director , Toxicology Program, Department of Environmental Health, School of Public Health and Community Medicine, University of Washington
1986-1992	Associate Professor , Department of Environmental Health, School of Public Health and Community Medicine, and Institute for Environmental Studies, College of Arts and Sciences, University of Washington
1979-1986	Assistant Professor , Department of Environmental Health, School of Public Health and Community Medicine, and Institute for Environmental Studies, College of Arts and Sciences, University of Washington
1978-1979	Postdoctoral Fellow in Toxicology, Department of Pharmacology, University of Kansas Medical Center, Kansas City, KS
1974-1978	Predoctoral Trainee , US Public Health Service, Department of Pharmacology, University of Kansas Medical Center, Kansas City, KS
1974	Research Assistant , Dept. Chemistry, Montana State University, Bozeman, MT

5. HONORS, AWARDS, FELLOWSHIPS,

Excellence in Pharmacology/Toxicology Award, PhRMA Foundation, 2015
 Public Communications Award, Society of Toxicology, 2014
 PANWAT Achievement Award, Pacific Northwest Society of Toxicology, 2014
 Elected Member, Institute of Medicine (now National Academy of Medicine), National Academies of Science Engineering and Medicine, 2011
 Elected Fellow, Washington Academy of Sciences, 2011
 National Associate, National Academy of Sciences, selected 2004 (lifetime membership)
 Fellow (Elected), Academy of Toxicological Sciences, 2000
 Fellow, International Union Against Cancer, 1996

Fellow (elected), American Association for the Advancement of Science, 1995
Zeneca Traveling Lectureship Award (Society of Toxicology), 1995
Achievement Award, Society of Toxicology, 1993
Rohm & Haas Distinguished Professor of Public Health Sciences, 1992-97
NIH Pharmacology Research Associate Program Postdoctoral Fellowship Award, 1979-1981 (declined)

6. PROFESSIONAL ACTIVITIES (OUTSIDE OF UW)

A. Professional Organizations

International Society for the Study of Xenobiotics (ISSX), 1992-present
American Association for Cancer Research, 1988 – present
Molecular Epidemiology Group, 1999-present
Sigma Xi 1987-present
Pacific Northwest Association of Toxicologists (Northwest Chapter, SOT)
Society of Toxicology, 1984-present
American Association for the Advancement of Science, 1977-present

B. Scientific Advisory Boards and Panels - National

Member, Board of Scientific Councilors, National Toxicology Program, 2019-2022
Member, Board of Trustees, Health and Environmental Sciences Institute (HESI), 2018-present
Review Coordinator, Review of Report and Approach to Evaluating Long-Term Health Effect in Army Test Subjects”, NASEM/BEST report, March, 2018
Chair, National Toxicology Program Peer Review Panel, NTP Technical Reports on Cell Phone Radiofrequency Radiation Studies. March 21-23, 2018, NIEHS, RTP, NC.
Reviewer, NASEM report Review of Advances Made to the IRIS Process, March 2018
Chair, NAS/NAM/NRC, Committee on the Review of the Health Effects of Electronic Nicotine Delivery Systems, 2017.
Member, Search Committee, Director of the Division of the National Toxicology Program (NTP), NIEHS, Research Triangle Park, NC, October, 2016-
Review Coordinator, Protocols for Conducting Systematic Reviews of Selected Endocrine-Disrupting Chemicals, National Academy of Sciences / National Research Council, 2016
Member, Society of Toxicology Foundation Endowment Board, 2015-
Review Coordinator, Styrene Assessment in the NTP 12th Report on Carcinogens, National Research Council, 2014-15
Member, University of North Carolina-Chapel Hill Gillings School of Public Health, External Advisory Committee, 2014, 2015
Member, Governmental and Regulatory Affairs Committee, Council of Graduate Schools, Washington, DC 2012-present
Member, Board of Trustees, Health and Environmental Sciences Institute (HESI) 2014-2015

Member- National Advisory Environmental Health Sciences Council (NIH/NIEHS Council), 2013-2017.

Member, External Science Advisory Board, Center for Research on Environmental Disease, Texas A&M University/Baylor College of Medicine, Houston, TX, 2014-2016.

Member, NAS/NRC Committee to Review the EPA IRIS process, 2012-2014.

Chair, Celebrating Member Accomplishments Task Force, Society of Toxicology, 2011-2014

Member, Planning Committee, Future of Toxicology II Symposium Society of Toxicology, 2013-14

Member, NAS/NRC Committee on the Future of Science at the EPA, 2011-2012.

Chair, Research Committee, Health Effects Institute, Boston, MA, 2010-present

Member, External Science Advisory Board, Fundamental & Computational Sciences Directorate, Pacific Northwest National Laboratory, Richland, WA; 2011 - 2015.

Member, NAS/ Institute of Medicine committee on "Breast Cancer and the Environment: The Scientific Evidence, Research Methodology, and Future Directions", 2010-2012.

External Reviewer, Cancer Research-United Kingdom; review of Cancer Center at University of Dundee, Scotland, June 17-18, 2010

Review Coordinator, NAS/IOM/NRC NRC Study on Toxicity Pathway-Based Risk Assessment: Preparing for a Paradigm Change. 2010.

Review Coordinator, NAS/IOM/NRC, Review of EPA's Draft IRIS Assessment of Tetrachloroethylene (BEST-K-06-03-A), 2009 – 2010.

Chair, Audit Committee, Society of Toxicology, 2012 (member, 2010-11).

Member (elected), Nominating Committee, Society of Toxicology, 2005-06; 2007-08

Chair, NAS/NRC Committee for Review of the Federal Strategy to Address Environmental, Health, and Safety Research Needs for Engineered Nanoscale Materials, 2008

Member, 50th Anniversary Planning Task Force, Society of Toxicology, 2007-2009

Member, External Scientific Advisory Board, Semiconductor Industry Association, 2005-2009

Chair, External Science Advisory Board, Procter and Gamble, Central Product Safety Division, Miami Valley Laboratories, Cincinnati, OH, 2003-2012

Member, External Science Advisory Board, University of Montana Environmental Health Sciences Center, Missoula, MT, 2003-2008

Member, External Science Advisory Board, Harvard University Environmental Health Sciences Center, 2003- 2014

Chair, External Science Advisory Board, NCRR Institutional Development Award, University of Alaska BRIN/INBRE program, 2001-2013

Member, External Science Advisory Board, Center for Research on Environmental Disease, University of Texas-MD Anderson Cancer Center, Smithville, TX, 1998-2011.

Editorial Board, Chemico-Biological Interactions, 1998-present; Toxicology Open journal, 2007-present.

Board of Trustees, Academy of Toxicological Sciences, 2005-08

Chair, Society of Toxicology Task Force on NIH Grant Reviews, 2004-2007

Board of Directors, Toxicology Education Foundation, 2004-08; Vice President, 2005-06

Member, Search Committee, NIEHS Extramural Program Director, 2008

Member, Molecular Epidemiology Working Group Program Committee, AACR, 2007
Member, NIH/NIEHS Special Emphasis Panel, ONES program review, 2007
Member, External Science Advisory Committee, CIIT Centers for Health Research, 2005-2006
Chair, NAS/NRC IOM Committee on Assessment of the Health Implications of Exposure to Dioxins, 2004-06.
Member, external review team for the National Center for Toxicogenomics, NIEHS, 2005.
Past-President, Society of Toxicology, 5/1/02-4/30/03 (President, 5/1/01-4/30/02; Vice-President, 5/1/00-4/30/01, Vice-President-elect, 5/1/99-4/30/00).
Chair, NAS/NRC Committee on Emerging Issues and Data on Environmental Contaminants, 2002-2005
Member, Working Group on Gene-Environmental Interactions, National Children's Study, NIH, 2001-04.
Ad hoc member, NIH Study Section, Epidemiology of Cancer-2 (EDC-2), June, 2003, March 2004
Member, External Science Advisory Board, University of New Mexico Environmental Health Sciences Center, 2003-2005.
Member, External Science Advisory Board, NIEHS Center for Environmental Health Sciences, University of California-Davis, 1999-2005
Member, External Science Advisory Board, NIEHS Center for Rural Environmental Health, Texas A&M University, College Station, TX, 2001-2005
Reviewer, Cancer Research –United Kingdom program grant, University of Dundee, Scotland, 2004
Councilor, International Union of Toxicology (IUTOX), 2001-04
Organizing Committee, International Conference on Molecular and Genetic Epidemiology of Cancer, AACR- Molecular Epidemiology group, 2002-03
Member, Board of Publication, Society of Toxicology, 2001-02
Member, Society of Toxicology Task Force on Professional Society Liaison Roles, 2004.
Member, Finance Committee, Society of Toxicology, 2001-02
Member, NIH Center for Scientific Review, Digestive Disease Boundary Review Panel, 2002.
Member, Scientific Review Panel, Agricultural Health and Safety Centers, NIOSH, 2001.
Member, NAS/NRC Panel on Arsenic in Drinking Water, 2001
Member, AAAS Education Sub-Committee, Court Appointed Scientific Experts (CASE), 2000-01
Advisory Board, BSCS, Inc, (Biological Sciences Curriculum Study), Colorado Springs, CO, 1999-2000
External Reviewer, Environmental Health Sciences Initiative, Battelle Pacific Northwest National laboratory, Richland, WA, 1999-2000
Member, Environmental Genome Working Group, NIEHS, 1997-98
Liaison, NRC/BEST Subcommittee on Arsenic in Drinking Water, 1997-98
Councilor, Mechanisms Specialty Section, Society of Toxicology, 1997-99
Member, Board of Environmental Studies and Toxicology, National Academy of Sciences/National Research Council, 1996-99
Secretary, Society of Toxicology, 1996-98 (Secretary-elect, Society of Toxicology, 1995-96)
Editor, SOT Communiqué, 1997-98

Visiting Scientist, International Agency for Research on Cancer (IARC), Lyon, France, 9/1/95-12/1/96.
President, Mechanisms Specialty Section, Society of Toxicology, 1996-97
Associate Editor, Toxicology and Applied Pharmacology, 1996-99
Editorial Board, Oncology Reports, 1994-97
Editorial Review Board, Environmental Health Perspectives, 1993-97.
Member, Executive Committee, International Congress on Toxicology VII, 1993
Chairman, Local Arrangements Committee, International Congress on Toxicology VII, 1992
External Grant Reviewer, Hong Kong Research Grants Council, University and Polytechnic Grants Committee, Hong Kong, 1992-93, 95-96, 99
Member, NIEHS Site Visit Team and Reviewer, Program Project Grant review, Oregon State University, 1992, 1993.
Editorial Board, Environmental Carcinogenesis & Ecotoxicology Reviews, 1992-present
Treasurer, American Board of Toxicology, Inc., 1991-94
Member, Science Advisory Board, The Institute for Wildlife and Environmental Toxicology, Clemson University, Clemson, SC. 1990-94; Chairman, 1993.
Councilor, Mechanisms Specialty Section, Society of Toxicology, 1990-93
Chairman, Membership Committee, Society of Toxicology, 1992-93
Membership Committee, (elected committee), Society of Toxicology, 1990-93
Board of Directors, American Board of Toxicology, Inc., 1990-94
Board of Publications, Society of Environmental Toxicology and Chemistry, 1989-91
Editorial Board, Toxicology and Applied Pharmacology, 1989-1996
Invited Participant, United States - Japan Cooperative Program on Development & Utilization of Natural Resources, Joint panel on toxic microorganisms symposium on "Cellular and Molecular Mode of Action of Selected Microbial Toxins in Foods and Feeds," National 4-H Center, Chevy Chase, MD, Oct. 31- Nov. 2, 1989
Member, Society of Toxicology ad hoc "Tox-90's Education Committee," 1988-1991
Invited Participant, Advisory workshop on significance and utilization of SARA Title III data, sponsored by USEPA, Chemical Manufacturer's Association and the National Academy of Sciences, NAS Headquarters, Washington, DC, Oct. 18-19, 1988
Participant, 1987 Gordon Research Conference on Mechanisms of Toxicity, Kimball Union Academy, Meriden, New Hampshire, July 27-31, 1987
Member, NIH Ad Hoc Scientific Review Panel for NIEHS contract on "Methods development for in vitro human metabolism of xenobiotics," February 19-21, 1985, Research Triangle Park, NC
Committee on Public Communications, Society of Toxicology, 1984-1985

C. Scientific Advisory Boards and Panels – Community and State (excluding UW)

Chair, Membership Committee, Washington State Academy of Sciences, 2017-18
Co-Chair, Membership Committee, Washington State Academy of Sciences, 2016-17
Chair, Membership Committee, Section 4, Washington State Academy of Sciences, 2013-15

Member, Board of Directors, Washington State Academy of Sciences, 2012-18

Member, Board of Directors, Global Health Research Fund, (Gubernatorial appointment) 2011-2015

Member, Board of Directors, CARE Northwest, 1998-2008

Chair, Tobacco Control Advisory Committee, American Lung Association of Washington, 2002-2007

Member, Grant Advisory Committee, American Lung Association of Washington, 2003-2006

Member, Board of Trustees, Seattle Biomedical Research Institute (SBRI), 2004-2006

Member, Tobacco Control Advisory Committee, American Lung Association of Washington, 2000-02

Member, Advisory Committee for Risk Management Conference, Washington Foundation for the Environment, 1992-93.

Pacific Northwest Association of Toxicologists (Northwest Chapter, SOT); founding member, 1984-present, Vice President, 1984-1985; President, 1987-88, Councilor, 1988-89; Councilor, 1997-98

Member, Scientific Advisory Board, State of Washington Initiative For Chemically-Related Illness, Washington State Department of Labor and Industries, 1997

Member, Northwest Consumer Food Safety Council, 1990-94

Chairman, Science Advisory Board (5 member board established by Initiative, pre-empting above SAB), Washington State Department of Ecology, 1989-1991; Member 1989-92.

Member, Science Advisory Board (14 member board established under legislative act), Washington State Department of Ecology, 1988-90

Member, Technical Work Group on Environmental/Regulatory Policy for the Puget Sound Water Quality Authority, 1987

Site Visitor, Western Washington University, Bellingham, WA; Requested to evaluate current occupational health and safety practices relating to toxic chemical storage and use at Western Washington University. Provided written report to Dean of the Graduate School, October 8, 1986

Member, Mayor's Advisory Task Force on PCB's, 1984-86

Member, Advisory Task Force to Assess Health Impacts of Sediment and Shellfish pollution at Eagle Harbor, WA. For Coalition of County and State Health Departments. October 1984-85

Member, Mayor's Health Advisory Panel on Gas Works Park, 1984

Member, Gypsy Moth Advisory Committee, Department of Agriculture, Washington State Medical Association, and Office of the Mayor, City of Seattle, 1983

Member, Emergency Insect Criteria Committee, State Department of Agriculture, 1982-86

Member, Washington State Pesticide Advisory Board (Gubernatorial Appointment), 1982-1985, reappointed 1986-1989

Member, State Department of Social and Health Services, *Ad Hoc* Committee to Review Health Implications of Totally Recycled Water Systems, 1981

D. Manuscript Referee:

Aquatic Toxicology; Archives of Environmental Contamination and Toxicology; Biochemical Pharmacology; Biochemistry; ; Cancer Epidemiology, Biomarkers & Prevention; Cancer Research; Carcinogenesis; Chemico-Biological Interactions; Drug Metabolism & Disposition; Environmental Science and Technology; Fundamental and Applied Toxicology; Journal of Pharmacology and Experimental Therapeutics; Molecular Pharmacology Nature Reviews-Cancer; Proceedings of the National Academy of Sciences; Pharmacology & Toxicology; Toxicology and Applied Pharmacology; Toxicological Sciences.

7. BIBLIOGRAPHY

(*Indicates students, post-doctoral fellows or research technologists under the direction of Dr. Eaton).

A. RESEARCH PAPERS IN REFEREED JOURNALS

1. **Eaton DL**, Klaassen CD. Effects of acute administration of taurocholic and taurochenodeoxycholic acid on biliary lipid excretion. *Proc Soc Exp Biol Med* 151:198-202, 1975.
2. **Eaton DL**, Poisner AM. Plasma pseudorenin in rats after alteration in the renin-angiotensin system. *Proc Soc Exp Biol Med* 154:6-8, 1977.
3. **Eaton DL**, Klaassen CD. Carrier-mediated transport of ouabain in isolated hepatocytes. *J Pharmacol Exp Ther* 285:480-488, 1978.
4. Iwamoto I, **Eaton DL**, Klaassen CD. Uptake of morphine and nalorphine by isolated rat hepatocytes. *J Pharmacol Exp Ther* 206:181-190, 1978.
5. **Eaton DL**, Klaassen CD. Carrier-mediated transport of the organic cation procaineamide ethobromide by isolated rat liver parenchymal cells. *J Pharmacol Exp Ther* 206:595-606, 1978.
6. Iga T, **Eaton DL**, Klaassen CD. Uptake of unconjugated bilirubin by isolated rat hepatocytes. *Am J Physiol* 236:C9-C14, 1979.
7. **Eaton DL**, Klaassen CD. Effects of microsomal enzyme inducers on carrier-mediated transport systems in isolated rat hepatocytes. *J Pharmacol Exp Ther* 208:381-385, 1979.
8. **Eaton DL**, Klaassen CD. Effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin, kepone and polybrominated biphenyls on transport systems in isolated rat hepatocytes. *Toxicol Appl Pharmacol* 51:137-144, 1979.
9. **Eaton DL**, Stacey NH, Wong KL, Klaassen CD. Dose-response effects of various metal ions on rat liver metallothionein, glutathione, heme oxygenase and cytochrome P-450. *Toxicol Appl Pharmacol* 55:393-402, 1980.
10. **Eaton DL**. Biliary excretion of 2,4,5-trichlorophenoxyacetic acid in the rat. *Toxicol Lett* 14:175-181, 1982.
11. **Eaton DL**, *Toal BF. Evaluation of the Cd/hemoglobin affinity assay for the rapid determination of metallothionein in biological tissues. *Toxicol Appl Pharmacol* 66:134-142, 1982.

12. **Eaton DL**, *Toal BF. A simplified method of quantitating metallothionein in biological tissues. *J Sci Total Environ* 28:375-384, 1983.
13. *Carpenter LA, **Eaton DL**. The disposition of 2,4-dichlorophenoxyacetic acid (2,4-D) in the rainbow trout, *Salmo gairdneri*. *Arch Environ Contam Tox* 12:162-173, 1983.
14. *Stinson MD, **Eaton DL**. Concentrations of lead, cadmium, mercury and copper in the crayfish (*Pacifasticus lenisculus*) obtained from a lake receiving urban runoff. *Arch Environ Contam Toxicol* 12:693-700, 1983.
15. Woods JS, Fowler BA, **Eaton DL**. Studies on the mechanisms of thallium-mediated inhibition of hepatic mixed function oxidase activity: Correlation with inhibition of NADPH Cytochrome c (P-450) reductase. *Biochem Pharmacol* 33:571-576, 1984.
16. Woods JS, **Eaton DL**, *Lukens C. Studies on porphyrin metabolism in the kidney: Effects of trace metals and glutathione on renal uroporphyrinogen decarboxylase. *Mol Pharmacol* 26:336-341, 1984.
17. Kalman D, **Eaton DL**, Schumacher RS, Covert D. Biological availability of lead in a paint aerosol. I. Physical and chemical characterization of a lead paint aerosol. *Toxicol Lett* 22:301-306, 1984.
18. **Eaton DL**, Kalman DA, *Garvey D, Morgan M, Omenn GS. Biological availability of lead in a paint aerosol. 2. Absorption, distribution and excretion of intratracheally instilled lead paint particles in the rat. *Toxicol Lett* 22:307-313, 1984.
19. **Eaton DL**. *Short Communications*: Effects of various trace metals on the binding of cadmium to rat hepatic metallothionein determined by the Cd/hemoglobin affinity assay. *Toxicol Appl Pharmacol* 78:158-162, 1985.
20. *Monroe DH, *Holeski CJ, **Eaton DL**. Effects of single dose and repeated dose pretreatment with 2(3)-*tert*-butyl-4-hydroxyanisole (BHA) on the hepatobiliary disposition and covalent binding to DNA of Aflatoxin B₁ in the rat. *Food Chem Toxicol* 24:1273-1281, 1986.
21. **Eaton DL**, *Richards JA. Kinetic evaluation of carrier-mediated transport of ouabain and taurocholic acid in isolated rat hepatocytes. Evidence for independent transport systems. *Biochem Pharmacol* 35:2721-2725, 1986.
22. *Holeski CJ, **Eaton DL**, *Monroe DH, *Bellamy GM. Effects of phenobarbital on the biliary excretion of Aflatoxin P₁-glucuronide and AflatoxinB₁-S-glutathione in the rat. *Xenobiotica* 17:139-153, 1987.
23. *Monroe DH, **Eaton DL**. Comparative effects of butylated hydroxyanisole (BHA) on the *in vivo* and *in vitro* biotransformation of Aflatoxin B₁ (AFB) in rat and mouse. *Toxicol Appl Pharmacol* 90:401-409, 1987.
24. Geraci JP, Dunston SG, Jackson KL, Mariano MS, *Holeski C, **Eaton DL**. Bile loss in the acute intestinal radiation syndrome in rats. *Rad Res* 109:47-57, 1987.
25. **Eaton DL**, *Monroe DH, *Bellamy G and Kalman DA. Identification of a novel dihydroxy-metabolite of aflatoxin B₁ formed both *in vivo* and *in vitro* in rats and mice. *Chem Res Toxicol* 1:108-114, 1988.

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28. *Ramsdell HS and **Eaton DL**. Modification of aflatoxin B₁ biotransformation *in vitro* and DNA binding *in vivo* by dietary broccoli in rats. *J Toxicol Environ Health* 25:269-275, 1988.
29. **Eaton DL**, *Stapleton PL. Simultaneous determination of cytosolic glutathione S-transferase and microsomal epoxide hydrolase activity toward benzo[a]pyrene-4,5-oxide by high performance liquid chromatography. *Analyt Biochem* 178:153-158, 1989.
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31. *Ramsdell HS, **Eaton DL**. Mouse liver glutathione S-transferase isoenzyme activity toward aflatoxin B₁-8,9-epoxide and benzo[a]pyrene-7,8-dihydrodiol-9,10-epoxide. *Toxicol Appl Pharmacol* 105:216-225, 1990.
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34. *Trenga CA, Kunkel DD, **Eaton DL**, Costa LG. Effect of styrene oxide on rat brain glutathione. *Neurotoxicology* 12:165-178, 1991.
35. *Chen ZY, **Eaton DL**. Differential regulation of cytochrome P450 IIB₁/2 by phenobarbital in hepatocellular hyperplastic nodules induced by aflatoxin B₁ or diethylnitrosamine plus 2-acetylaminofluorene in male F344 rats. *Toxicol Appl Pharmacol* 111:132-144, 1991.
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41. *Hamel DM, *White C, **Eaton DL**. Determination of γ -glutamylcysteine synthetase and glutathione synthetase activity by HPLC. *Toxicology Methods* 1:273-288, 1992.
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56. *Gallagher EP, Wienkers LC, *Stapleton PL, Kunze KL, **Eaton DL**. Role of CYP4501A2 and CYP4503A4 in the bioactivation of aflatoxin B₁ (AFB₁) by human liver microsomes. *Cancer Research* 54: 1-8, 1994.
57. *Chen Z-Y, Liu Y-F, *He C-Y, *White CC, **Eaton DL**. Inhibition of cell proliferation by ciprofibrate in GST - Positive rat hepatic hyperplastic nodules, *Cancer Research* 54: 2622-2629, 1994.
58. Kavanagh TJ, Raghu G, *White CC, Martin GM, Rabinovitch PS, **Eaton DL**. Enhancement of glutathione content in glutathione synthetase-deficient fibroblasts from a patient with 5-oxoprolinuria via metabolic cooperation with normal fibroblasts. *Exp. Cell Res.* 212: 69-76, 1994.
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68. *Buetler TM, *Bammler T., Hayes JD, and **Eaton DL**. Oltipraz-mediated changes in aflatoxin B₁- biotransformation in rat liver: Implications for human chemointervention. *Cancer Research*, 56: 2306-2313, 1996.

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88. *Kelly EJ, Erickson KE, Sengstag C and **Eaton, DL**. Expression of human microsomal epoxide hydrolase in *Saccharomyces cerevisiae* reveals a functional role in aflatoxin B₁ detoxification. *Toxicol Sci* 65:35-42, 2002.
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131. Parent R, **Eaton DL** and Goldstein BD. Toxicology in the courtroom: Establishing 'Causation'- a Roundtable Discussion. *Toxicological Sciences* 90: S1, #717, 2006.
132. *Gross-Steinmeyer K, Zhu C, *Stapleton PL, *Tracy JH, *Bammler TK, Strom SC, Thummel KT and **Eaton DL**. Down-regulation of CYP3A4 in human primary hepatocytes and human LS180 colorectal carcinoma cells by sulforaphane. *Toxicological Sciences* 90: S1, #1815, 2006.
133. Maurissen JT, *McHugh T D, **Eaton D**, Ehrich M, Hoberman A, and Reid L. Approaches to manage ethical conflicts of interest (Workshop). *Toxicological Sciences* 96(1): #647, 2007.
134. *Poulton EJ, **Eaton DL**, Zhou Z, Thummel KT and Bammler TK. The isothiocyanate moiety is required for sulforaphane (SFN)-mediated inhibition of ligand activation of the human Steroid and Xenobiotic Receptor (SXR). *Toxicological Sciences* 96(1): #2128, 2007.
135. **Eaton, DL** (Chair). New Concepts in the Etiology of Breast Cancer: From Genes to Environment and Back Again (SOT Symposium). *Toxicological Sciences* 102(1): #641, 2008
136. *Peck EC, P. L. Stapleton PL, Grollman AP and **Eaton DL**. Activation of aristolochic acid to mutagenic metabolites by human CYPs 1A1, 1A2 and 3A4. *Toxicological Sciences* 102(1): #6760, 2008.
137. *Poulton EJ and **Eaton DL**. Mutations in the PXR gene affect basal expression and inducibility but do not prevent the ability of sulforaphane to inhibit ligand binding to PXR. *Toxicological Sciences* 102(1): #6768, 2008.
138. Smith WE, Lin T, Tracy JH, White CC, Xiaoge H, Xiaohu G, **Eaton DL** and Kavanagh TJ. Uptake of quantum dots into cultured human hepatocytes. Pacific Northwest Association of Toxicologists Annual Meeting 2009, Seattle, WA, September. Poster presentation.

139. Smith WE, Tracy JH, White CC, Bammler TK, Xiaoge H, Xiaohu G, Kavanagh TJ and **Eaton DL**. 2009. Disposition of quantum dots in HepG2 cells and primary human hepatocytes. Society of Toxicology Annual Meeting, Baltimore, MD, March. Poster presentation.
140. Smith WE, Lin T, Tracy JH, White CC, Xiaoge H, Xiaohu G, **Eaton DL** and Kavanagh TJ. Effects of Quantum Dots on Cellular Stress Markers in HepG2 Cells. Society of Toxicology Annual Meeting, Salt Lake City, UT, March. SOT Abstracts 2010, #374.
141. *Smith WE, Ashfarinajad Z, Hu X, Gao X, **Eaton DL** and Kavanagh TJ. Gene expression changes induced by polymer-coated quantum dots in HepG2 cells. Pacific Northwest Association of Toxicologists Annual Meeting 2011, Bonnevill Hot Springs, WA. Poster Presentation.
142. *Smith WE, White CC, Brownell JL, Ashfarinajad Z, Hu X, Gao X, Polyak SJ, **Eaton DL**, and Kavanagh TJ. Uptake of polymer-coated quantum dots by Kupffer cells promotes a pro-inflammatory response. NCNHIR Consortium Meeting 2011, Seattle, WA.
143. *Smith WE, Brownell JL, Ashfarinajad Z, Hu X, Guo, X, Polyak SJ, **Eaton DL** and Kavanagh TJ. Polymer-coated quantum dots elicit a pro-inflammatory response in primary human hepatocyte cultures. Society of Toxicology Annual Meeting, Washington, DC, March. SOT Late-breaking Abstracts 2011, #2781.
144. *Smith WE, White CC, Brownell JL, Ashfarinajad Z, Hu X, Gao X, Polyak SJ, **Eaton DL**, and Kavanagh TJ. Differential gene expression changes in two human hepatocyte model systems to quantum dot exposure. Society of Toxicology Annual Meeting, San Francisco, CA, March. SOT Abstracts 2012, #1267.
145. *Smith WE, White CC, Brownell JL, Ashfarinajad Z, Hu X, Gao X, Polyak SJ, **Eaton DL**, and Kavanagh TJ. Uptake of polymer-coated quantum dots by Kupffer cells promotes a pro-inflammatory response. NCNHIR Consortium Meeting 2012, Research Triangle Park, NC.
146. **Eaton, DL**, and Tsuji, J. Breast Cancer and the Environment: Interaction of genetics, lifestage and the environment. *Society of Toxicology Annual Meeting, San Francisco, Session Chair*. Abstract # 804, 2012.
147. *Schaupp CM, Bammler TK, Byer RP, Terrance J. Kavanagh TJ and **Eaton DL**. Absence of Upstream Consensus Regulatory Element Sequences for Nrf2 in Human Glutathione S-Transferase Genes. *The Toxicologist*, #2025, 2013.
148. *Ward T, R. S. McMahan RS, White CC, Shang J, Hu X, Gao X, **Eaton DL**, Kavanagh TJ, Parks WC and Altemeier WA. *The Toxicologist* #419, 2014.
149. *Chang S, Voellinger, JL, White C, Kelley EJ, and **Eaton DL**. A Tissue-Engineered Rat/Human Liver Microphysiological System for Drug and Chemical Testing. *The Toxicologist* #708, 2015.
150. *Cook1 TJ, Hoekstra JG, Stewart T, Canales KK, Ho P, Salvador AA, Gonzalez-Cuyar LF, Nelson G, Racette BA, Checkoway H, **Eaton DL** and Zhang J, Astroglial Mortalin Is Decreased in the Striatum of Manganese-Exposed Mine Workers and Enhances Neurotoxicity, *The Toxicologist* #2352, 2016.
151. *Chang S-Y, Weber E, Kelly EJ, **Eaton DL** and Neumann T. Microphysiological Systems (MPS) to Identify Organ-Organ Interactions in Toxicology: Hepatic Metabolism Enhances Nephrotoxicity of Aristolochic Acid, *The Toxicologist* #1105, 2016

152. Eaton DL . Public Health Consequences of E-cigarettes: A Focus on Special Concerns for Youth and Young Adults . Symposium, Society of Toxicology, The Toxicologist, 168, p 501, #3232, 2019.

8. PATENTS AND OTHER INTELLECTUAL PROPERTY

License for rabbit polyclonal antibody towards human Catechol –O-Methyltransferase, UW TechTransfer, 2001.

Patent application # 11/867,299. Sulforaphane and structural analogs as antagonists of the human Pregnane X Receptor (PXR), 2007.

9. FUNDING HISTORY:

A. Active Grants

NONE – Retired from academic research on March 15, 2019.

B. Completed Grants, 1985-2019

(DL Eaton, PI – excludes institutional and co-Investigator grants)

NIH 5-P30 ES07033 (Kavanagh, Center Director) 04/01/16 - 03/31/20 0.6 Cal Mo. FTE

PI: Terrance J. Kavanagh (2013-2019)

Title: Center for Exposures, Diseases, Genes & Environment (NIEHS Center Administrative Core)

Role: PI 1995-2013; Associate Director, 2013 - present. The major goal of this NIEHS Center Grant is to provide core support to enhance multidisciplinary collaborations among approximately 75 established investigators in the School of Public Health, School of Pharmacy, and the School of Medicine who are investigating the biochemical and molecular basis for human diseases with an environmental etiology.

EPA-G2013-STAR-L1 (Faustman) 12/1/2014 – 11/30/2018 0.6 Cal Mo FTE

Environmental Protection Agency \$6,000,000

Title: Predictive Toxicology Center for Organotypic Cultures and Assessment of AOPs for Engineered Nanomaterials

Role: Co-PI on liver microphysiological systems project. The overall goal of this Center is to develop innovative organotypic culture systems to better evaluate the potential for cellular and organ toxicity following exposure to Engineered Nanomaterials (ENMs) within an adverse outcomes pathway (AOP) model.

NSF DGE-1256082 \$ 34,227,534 7/29/2015 – 7/31/2018 no FTE

Title: Graduate Research Fellowship Program (GRFP)

Role: Institutional PI

The purpose of this amendment is to support the NSF graduate Fellows on tenure and on partial tenure at the University of Washington.

NIH UH2/UH3 7/1/2012-6/30/2017 0.6 Cal Mo FTE

PI: Jonathan Himmelfarb

Title: Integrated Microphysiological Systems For Drug Efficacy and Toxicity Testing in Human Health And Disease: Microphysiological Systems – Kidney

Role: co-Investigator on UH3 portion. This grant will develop the Nortis microphysiological system to utilized human kidney cells to study drug toxicity and efficacy.

U19 RFA ES -09-011

10/01/2010-9/30/2015

1.8 Cal Mo FTE

PI: Program Director, Terrance Kavanagh

Title: Linking the physical and chemical characteristics of Qdots to their toxicity.

Role: co-PI (with Bill Parks) PI for Project 1 – in vitro toxicology. This consortium program will identify physical and chemical characteristics of quantum dot (Qdot) nanoparticles associated with toxicity to cells and mice using genetic and epigenetic analyses. The In vitro Studies project will correlate the physical and chemical characteristics of quantum dots with quantitative measures of cytotoxicity in human- and mouse-derived model cell systems.

1 R01 GM079280-01A1 (D. Eaton, PI)

9/01/07 – 8/31/11

1.2 Person Months

NIH/NIGMS/NIEHS/NCI \$200,000 ADC

Isothiocyanates as specific antagonists of human SXR (currently on one year no cost extension)

Puget Sound Partners for Global Health

07/01/07 – 06/30/08

0.6 Person Months

PSPGH 2007-55, (D. Eaton, PI) \$70,000 ADC

Preventing drug-drug interactions in the treatment of TB in HIV/AIDS patients: an animal model

5R25 ES10738, (Eaton, PI) 09/30/00-08/31/08

NIH/NIEHS \$248,415 ADC

Environmental Health Sciences as an Integrative Context for Learning

1 U19 ES11387 (Eaton, PI) 09/30/01 - 08/31/08

NIH/NIEHS \$560,000 ADC

The FHCRC/UW Toxicogenomics Consortium.

R01 ES05780-01-17, David L. Eaton, PI: 08/01/87 - 07/31/04 (to 8/01/05);

NIH/NIEHS \$266,142 (ADC); \$1,584,405 (TDC)

Species differences in biotransformation of aflatoxin

P42 ES04696 (H. Checkoway, Program Director) 04/01/00 - 03/31/05

.5 % FTE

NIH/NIEHS; Award Amount - Total Program \$1,989,426 ADC

Superfund Basic Research Program: Effects-Related Biomarkers of Toxic Exposure

DL Eaton, Deputy Director; (Administrative Core)

P42 ES04696 (sub-project) H. Checkoway, Program Director, and Project PI 04/01/00 - 03/31/05

NIH/NIEHS; Award amount: \$156,501 ADC: co-investigator, sub-project); 5 %
Environmental and Biochemical Risk Factors for Parkinson's Disease

R01 ES10750 (H. Checkoway PI). Gene/Environment Interactions in Parkinson's Disease
09/01/00 – 06/30/05: co-Investigator; 5%; NIH / NIEHS.

P42 ES04696 Superfund Basic Research Program, DL Eaton, Program Director (04/01/90 – 09/30/99); ~ \$2 million ADC (H Checkoway assumed Program Director responsibilities in 1999; DL Eaton remained active as Deputy Director)

P42 ES04696 Superfund Basic Research Program – Project 1: Glutathione as a biomarker of toxic exposure”, DL Eaton, Project PI, 04/01-85 – 09/30/96. (TJ Kavanagh assumed PI responsibility in 1996; DL Eaton continued as co-investigator)

R01 AG17635, David L. Eaton, PI 06/15/99-06/30/04
NIH/NIA \$188,383 ADC; \$731,755 TDC
Significance of Genetic Variation in Estrogen Metabolism”.

R25 ES08030 DL Eaton, PI 08/01/96-07/30/00
NIH/NIEHS \$100,000 ADC
Project Greenskate Teacher Training and Dissemination

R01 ES03933 DL Eaton, PI 05/01/86-04/30/99
NIH/NIEHS \$150,000 - \$200,000 , ADC
Modification of Aflatoxin Disposition by Enzyme Inducers / Effect of Enzyme Inducers on Liver Preneoplastic Lesions,

R25 ES06938, DL Eaton, PI 07/01/94-06/30/97
NIH/NIEHS \$100,000 ADC.
Risky Business: Living in a Chemical World

10. CONFERENCES AND SYMPOSIUMS / INVITED PRESENTATIONS

Symposium Speaker, Society of Toxicology 58th Annual Meeting, “This Is Your Teen Brain on Drugs: In Search of Biomarkers Unique to Dependence Toxicity in Adolescents”. Topic: Public Health Consequences of e-Cigarettes, San Antonio, TX, March 18, 2019.

Seminar Speaker, SUNY-Stony Brook School of Medicine, “Using Microphysiological Systems to Identify ‘Organ-Organ Interactions’ in Toxicology: Effect of the Liver on Aristolochic acid Nephrotoxicity as a proof of concept”, June 25, 2016.

Invited Speaker, NIEHS-SOT 50th Anniversary Celebration, NIEHS, RTP, NC, July 13, 2016.

Kopriva Science Lecture, What Scientists Know- and Don't Know-About the Causes of Cancer; Montana State University, Bozeman, MT, Oct. 8, 2013.

Keynote Speaker, Health Effects Institute Annual Meeting, Boston, MA, Gene-Environment Interactions in Air Pollution Research: Challenges and Opportunities, May 2, 2011.

Kuna Distinguished Annual Lecture, Environmental and Occupational Health Sciences Institute, Rutgers's University/Robert Wood Johnson Medical School, Piscataway, NJ, May 6, 2010.

Invited Speaker, 10th Annual John Doull Symposium, University of Kansas Medical Center, *Modulation of Aflatoxin-DNA binding by phytochemicals in human hepatocytes* Sept. 3, 2009

Invited Speaker, NAS/IOM Workshop, *Environmental Health Science Decision Making: Risk Management, Evidence, and Ethics*, National Academy of Sciences, Washington DC. January 15, 2008,

Invited Representative (UW), Science and Technology in Society (STS) *forum*: "Harmony with Nature" and "Innovation", Kyoto, Japan, Oct. 7-9, 2007.

Invited Speaker, *The NAS and WHO on Dioxin and Dioxin Like Compounds: International Policy Implications and Potential Impact*, Michigan State University / NIEHS Superfund Basic Research Program Conference, Sept. 19, 2007.

Invited Participant, Western Library Association Annual Conference, 'Open Access', Tucson, AZ, Sept. 16-17, 2007.

Invited Speaker, NAS/NRC Workshop, Quantitative Approaches to Characterizing Uncertainty in Human Cancer Risk Assessment Based on Bioassay Results National Academy of Sciences, Washington DC, June 5, 2007.

Invited Speaker, Glutathione S-Transferases, Drug Metabolism short course, American Association of Pharmaceutical Scientists Annual Meeting, San Antonio, TX, Oct. 29, 2006.

Invited Speaker, Presidential Symposium, AAS Annual Meeting: "Risk-Risk Trade-offs: Public Health Examples- 'Contaminated' Drinking Water", ST. Louis, MO, February 18, 2006.

Seminar Speaker, University of Wisconsin-Madison, Interdisciplinary Toxicology program, Oct. 20, 2005.

Discussion Leader, National Institute of Environmental Health Sciences Strategic Planning meeting, Oct. 18-19, 2005.

Invited Speaker, "Functional Genomics and Public Health Protection in the 21st Century", 25th Anniversary Celebration of the National Toxicology Program, National Academy of Sciences, Washington, DC, May 11, 2005.

Organizer and Moderator, Issues Session, Society of Toxicology annual meeting; Reorganization of the NIH Grant review process and its potential impact on toxicology research and training. New Orleans, LA, March 9, 2005.

Invited Speaker, Agricultural Health Study Biomarker Workshop on Cancer Etiology, RTP, NC, sponsored by National Cancer Institute; March 2-3, 2005.

Invited Participant, Workshop on Genetics and Environmental Regulation, Arizona State University College of Law, Tempe, AZ, Jan. 13-14, 2005.

Invited Speaker, NAS/IOM Conference on Implications of Genomics for Public Health, "Gene x Environment Interactions", Oct. 7, 2004, National Academy of Sciences, Washington, DC.

Invited Speaker, North American Congress of Clinical Toxicology, “Liver Cancer”, Continuing Medical Education seminar on Insights into the Pathogenesis and Treatment of Toxin-induced Liver Disease”, Seattle, WA, Sept. 10, 2004.**Session Chair**, SOT/SETAC Pelston Workshop on “Emerging Molecular and Computational Approaches for Cross-Species Extrapolations”, held in Portland, OR July 18-22, 2004.

Symposium Chair, Novel approaches to engaging toxicologists in K-12 science education and outreach, Society of Toxicology Annual Meeting, Baltimore, MD, March 25, 2004.

Invited Commentary, NTP Board of Scientific Counselors Working Group on the National Toxicology Program Vision for the 21st Century, Baltimore, MD, March 25, 2004.

Workshop Speaker, Society of Toxicology Annual Meeting, “Working with Congress”, Baltimore, MD, March 23, 2004.

Public Speaker, What Scientists Know-and Don’t Know About the Causes of Cancer, Barrow, Alaska, Sponsored by Barrow Arctic Science Consortium; July 29, 2003.

Invited Participant, WHO/ICPS Planning Committee for ‘Environmental Health Criteria Document on Principles for Evaluating Health Risks in Children Associated with Exposure to Chemicals’, Seattle, WA July 24-25, 2003.

Invited Speaker, Science for Judges, “Scientific Judgment and Toxic Torts”, March 28, 2003, Brooklyn Law School, Brooklyn, New York.

Co-Organizer, Molecular and Genetic Epidemiology of Cancer, AACR/SOT sponsored meeting, “Impact of Molecular Epidemiology” (Session Chair), Jan 18-22, 2003, Kona, Hawaii.

Invited Speaker, “Toxicology Training in the 21st Century”, presented at a NIGMS Workshop on Pharmacological Sciences Training Program Needs, Bethesda, MD, 8/06/02

Seminar Speaker, University of New Mexico, School of Pharmacy, 4/22/02

Seminar Speaker, Columbia University School of Public Health, 4/16/02

Seminar Speaker, New York University, Nelson Institute for Environmental Medicine, 4/15/02

Sitlington Distinguished Lecturer in Toxicology, 2001, Oklahoma State University, “Molecular basis for species and interindividual differences in susceptibility to carcinogens: Aflatoxin B1 as an example”, Stillwater, OK 11/08-09/2001.

Welcome and Introduction, “Use of Genomic Information in Risk Assessment”, Current Concepts in Toxicology workshop, Bethesda, MD, Nov. 6-7, 2001.

Invited Speaker, American College of Occupational and Environmental Medicine annual meeting, “Use of genomic information in Environmental and Occupational Medicine”, Seattle, WA. Nov. 11, 2001

Invited Speaker, National Capitol Chapter, Society of Toxicology, “New Directions in Toxicology Education for the 21st Century”, Washington, DC, May 15, 2001.

Invited Speaker, “Understanding the Science of Toxicology”, for ‘New Directions in Expert Testimony: Scientific, Technical and Other Specialized Knowledge Evidence in Federal and State Courts’, American Law Institute-American Bar Association, San Francisco, CA, April 28, 2001.

Seminar Speaker, University of Colorado Health Sciences Center, “Molecular Basis for Species Differences in Aflatoxin Carcinogenesis”, Feb. 21-22, 2001.

Invited Speaker, “Environmental Pollution and Disease: Fact and Fiction”, Practical Primary Care Conference, Billings, MT, Oct. 27, 2000.

- Invited Speaker**, "Susceptibility to Disease and Human Genetic Variation: Implications of the Human Genome Project to the Practice of Medicine", Practical Primary Care Conference, Billings, MT, Oct. 28, 2000.
- Invited Speaker**, Interindividual Differences in Response to Chemoprotection Against Aflatoxin-Induced Hepatocarcinogenesis: Implications of Human Biotransformation Enzyme Polymorphisms", Sixth International Symposium on Biological Reactive Intermediates, Chemical and Biological Mechanisms In Susceptibility to and Prevention of Environmental Diseases, Paris, France, Université René Descartes, July 16-20, 2000.
- Invited Speaker**, "Genetics of Susceptibility to Environmental Factors", International Conference on Arctic Development, Pollution and Biomarkers of Human Health, Anchorage, AK April 30-May 3, 2000.
- Invited Discussant**, "Legal Liabilities at the Frontier of Predictive Genetic Testing", 2nd Annual Arizona State University-Smith Kline Beecham Conference on Genetics and the Law, Phoenix, AZ, April 7-8, 2000.
- Seminar Speaker**, University of Florida, The molecular Basis for species differences in carcinogenicity of the dietary carcinogen, aflatoxin B1, Jan. 21, 2000, Gainesville, FL.
- Invited Speaker**, 36th Annual Hanford Life Sciences Symposium, "Individual Susceptibility and Genetic Polymorphisms", Oct. 19, 1999, Richland, WA.
- Invited Speaker**, American Chemical Society Continuing Education Course, Principles of Toxicology, "Quantitation in Toxicology", and "Basic Principles and Approaches to Experimental Animal Testing in Toxicology", April 27, 1999, San Francisco, CA.
- Invited Speaker**, Society of Toxicology, High School Teachers Program, Paracelsus Goes to School, "Genes, The Environment and Cancer", March, 15, 1999, New Orleans, LA
- Invited Speaker**, Society of Toxicology Undergraduate Minority Student Program, "How Chemicals Act in the Body, March 14, 1999, New Orleans, LA
- Seminar Speaker**, University of Utah, "Molecular basis for species differences in aflatoxin carcinogenesis", November 23, 1998, Salt Lake City, UT.
- Invited Discussant**, "Genomic Research on Populations Exposed to Environmental Toxins: Ethical, Legal and Social Issues", Nov. 13-14, 1998, Boston, MA.
- Seminar Speaker**, University of Texas-Houston Medical Center, "Molecular basis for species differences in aflatoxin carcinogenesis", October 13, 1998, Houston, TX.
- Invited Speaker/Session Chair**, International Neurotoxicology Conference, Pesticides and Susceptible Populations, "Biotransformation Enzyme Polymorphisms and Pesticides Susceptibility", Sept. 13, 1998, Little Rock, AR.
- Invited Speaker**, Toxicology Forum, "Genetic Polymorphism in the human Glutathione S-transferases: Implications for Human Health", July 12-18, 1998, Aspen, CO.
- Invited Speaker**, American Chemical Society Continuing Education course "Toxicology for Chemists", San Francisco, CA; April 1-3, 1998.
- Symposium Chair**, Society of Toxicology Annual Meeting, "The Epidemiology of Breast Cancer: Unraveling the Roles of Genetics, Lifestyle and Environmental Factors", March 5, 1998.
- Invited Speaker**, "Cancer, Genes and the Environment", lecture in high school workshop "Paracelsus goes to school", Society of Toxicology Annual Meeting, March 2, 1998.

Invited Speaker, Society for Quality Assurance 13th annual meeting, "Liaison Organizations: Society of Toxicology", October 23, 1997, Seattle, WA.

Invited Speaker, NATO Advanced Studies Institute, "Molecular and Applied Aspects of Oxidative Drug Metabolizing Enzymes", August 31-Sept. 11, 1997, Antalya, Turkey

Seminar Speaker, Toxicology Training Program, Department of Environmental Medicine, University of Rochester, May 28, 1997

Symposium Co-organizer and Speaker, Society of Toxicology Annual Meeting, "Genetic Polymorphisms in the Glutathione-S-Transferases", March 10, 1997.

Invited Speaker, ILSI Health and Environmental Sciences Institute, 1997 annual meeting: Emerging Issues in Risk Assessment: Gene-Environment Interactions, Miami Beach, FL, Jan. 21, 1997

Invited Participant, NCI Workshop on Diet, Nutrition, Cancer and Genetic Susceptibility, Jan. 22-23, 1997, Washington, DC.

Plenary Speaker, American College of Veterinary Pathologists, Gene-Environment Interactions: Significance of Human Biotransformation Enzyme Polymorphisms, Dec. 6, 1996, Seattle, WA.

Symposium Speaker, ISSX Annual Meeting: Dietary Modulation of Glutathione S-Transferases, Oct. 20-24, 1996, San Diego, CA

Invited Speaker, Gordon Research Conference on Drug Metabolism, "Class alpha glutathione S-transferases: mechanisms and relevance to variations in human cancer risk", July 7-12, 1996, Holderness, NH.

Zeneca European Lecture Tour: "Molecular basis for species differences in susceptibility to aflatoxin carcinogenesis", University of London, London, England, 8/1/96; University of Dundee, Dundee, Scotland, 8/8/96; University of Newcastle, Newcastle-Upon-Tyne, England, 8/14/96; Medical Research Council/University of Leicester, Leicester, England, 8/16/96; Zeneca International, Macclesfield, England, 8/18/96; University of Basel, Basel, Switzerland, 9/22/96; Lilly Development Center, Brussels, Belgium, 10/2/96; TNO Nutrition and Food Research Institute, Zeist, The Netherlands, 10/6/96.

Invited Speaker, "The role of cytochromes P450 and glutathione S-transferases in species differences in carcinogen metabolism", International Symposium: Evaluation of Butadiene & Isoprene Health Risks, Blaine, WA, June 27-29, 1995

Invited Speaker, "Genetic polymorphisms as susceptibility factors to environmental pollutants", 47th Annual meeting, American Academy of Forensic Sciences, Medical Toxicology Section Workshop, Seattle, WA February 14, 1995

Seminar Speaker, "Molecular basis for species differences in aflatoxin carcinogenicity", Duke Marine Sciences Center, Beaufort, NC July 19, 1993

Seminar Speaker, "Molecular basis for species differences in aflatoxin carcinogenicity", National Institutes for Environmental Health Sciences, Research Triangle Park, NC, April 27, 1993

Seminar Speaker, Toxicology Scholars Colloquium, "Molecular basis for species differences in aflatoxin carcinogenicity", University of Connecticut, Center for Biochemical Toxicology, Feb. 26, 1993

Seminar Speaker, Toxicology Scholar Seminar Series, "Molecular basis for species differences in aflatoxin carcinogenicity", Duke University Integrated Toxicology Program, Feb. 22, 1993

- Seminar Speaker**, “Molecular basis for species differences in aflatoxin carcinogenicity”, University of California-Davis, Feb. 8, 1993
- Invited Speaker**, Rocky Mountain Academy of Occupational and Environmental Medicine Conference, “Risky Business: Clinical Toxicology & Environmental Health, and Relative Risk Assessment”, Jan. 8, 1993
- Seminar Speaker**, Molecular basis for species differences in aflatoxin carcinogenicity, Chemical Industry Institute for Toxicology, Research Triangle Park, NC, June 4, 1992
- Invited Speaker**, “Risk Assessment in Managing Environmental Exposure Communications and Media Relations”, Medical Group Management Association, Occupational Medicine Assembly Conference, Orlando, FL, February 18, 1992
- Invited Speaker**, “Principles of Toxicology; Metals Toxicology; Carcinogenesis, mutagenesis and Teratogenesis” lectures, as part of *Risk Assessment, Management and the Communication of Drinking water Contamination*, Continuing Education program sponsored by USEPA and NEHA, Portland, OR, June 27, 1991
- Invited Alumni Speaker**, “The biochemical and molecular basis for species differences in susceptibility to aflatoxin B₁ carcinogenesis”, Department of Pharmacology, Toxicology and Therapeutics, University of Kansas Medical Center, Kansas City, KS April 9, 1991
- Invited Seminar Speaker**, “The biochemical basis for species differences in aflatoxin carcinogenicity”, Department of Environmental Health Sciences, Johns Hopkins University School Hygiene and Public Health, Baltimore, MD, February 12, 1990
- Invited Seminar Speaker**, “The role of biotransformation in species susceptibility to aflatoxin B₁ carcinogenesis”, Medical Research Council, Toxicology Division, Carlshalton, England, Sept. 5, 1989
- Participant and Presenter**, Third International Conference on Glutathione S-Transferases, “Conjugation of aflatoxin B₁-8,9-epoxide by human liver glutathione S-transferases”, Edinburgh, Scotland, August 31 - September 3, 1989
- Invited Guest**, National Public Radio Broadcast, Discussion on Alar in Apples, June 21, 1989
- Invited Speaker**, “Pesticides in Our Food and Water. Putting the Risks in Perspective”, public information seminar sponsored by the National Environmental Health Association Annual Meeting, Seattle, WA June 25, 1989
- Invited Speaker**, “Approaches to Risk Assessment and Risk Communication for Forest Use Herbicides”, presented to Regional Forest Managers and other agency officials, Portland, Oregon, April 16, 1987
- Invited Speaker**, “Estimating Occupational and Public Health Risks of Pesticides”, Northwest Aerial Applicators Association Convention, Pendleton, OR, November 12, 1986
- Invited Speaker**, “Toxicology of Wood Preservatives: Pentachlorophenol, Creosote and Arsenicals”, given for Montana Department of Agriculture as part of wood preservative applicators' certification training program, Kalispell and Helena, MT, September 30 and October 1, 1986
- Session Co-Chairman**, “Human Health Implications of Contaminated Seafood”, International Symposium on Toxic Chemicals and Aquatic Life: Research and Management; provided closing remarks and session summary, Seattle, WA, September 16-18, 1986

Seminar Speaker, “Effects of enzyme induction on hepatobiliary disposition and DNA binding of Aflatoxin B₁ in the rat”, presented at Philadelphia College of Pharmacy and Science, Department of Pharmacology and Toxicology, October 12, 1984

11. UNIVERSITY SERVICE

A. Committees

Member, Executive Committee, President’s Population Health Initiative, 2016-18
Member, Activity Based Budget Steering Committee, 2016
Member, International Travel Risk Policy Advisory Committee, 2015-18
Member, Graduation and Retention Task Force, 2015-18
Member, CoMOTION (Formerly C4C) Advisory Council, 2015-18
Chair, Industrial Relations Oversight Committee, 2015-18
Chair, Graduate Tuition Policy Committee, 2014-18
Chair, Review Committee, Dean of the School of Law, 2014-15
Member, Faculty Salary Policy Committee, 2013-2016
Chair, UW Board of Environmental Health and Safety, 2013-2017
Member, President’s Task Force on Sexual Assault Prevention, 2013-16
Member, President’s Advisory Committee on Enterprise Risk Management (PACREM), 2013-2016
Member, Board of Deans and Chancellors, 2013-2018
Member, Office of Research Proposal Review Committee, 2012-2018
Member, Search Committee, Director, Health Sciences Administration, 2011-12
Chair, Search Committee, Director for School of Fisheries and Aquatic Sciences, 2011-12
Member, UW Ocean Observing Initiative (OOI) Regional Scale Node Advisory Group, 2010-12
Member, Animal Facilities Renovation Advisory Committee, 2011-2013
Member, institutional review committee for School of Nursing, 2011-2012
Member, Search Committee, Dean for School of Dentistry, 2011-2012
Member, Activity Based Budgeting Advisory Committee, 2010-2011
Member, Research Roadmap Oversight Group, 2009-2010
Chair, Department of Environmental and Occupational Health Sciences PhD Oversight Committee, 2009-2010
Chair, Reduced Appointment Policy Committee, 2009-2010
Member, Search Committee, Dean, College of the Environment, 2009-2010
Member, Search Committee, Vice Provost for Global Affairs, 2008
Member, Internal Advisory Board, NHGRI ‘Genes and Environment’ Statistical Coordinating Center (Bruce Weir, PI), 2008-2018
Member, Internal Advisory Board, NHGRI ELSI Center of Excellence, Center for Genomics & Healthcare Equality, 2006-2012 (PI, Wylie Burke)
Chair, Office of Research Proposal Review Committee, 2006-12

Member, External Advisory Board, Berman Environmental Law Clinic, UW School of Law, 2004-08

Member, Selection Committee, Outstanding Graduate Student Mentor Award, 2000-2006

Research Advisory Board, Office of the Provost, 1999-2006

Member, Public Health Genetics Program Executive Committee, 1999-present

Member, Scientific Advisory Board, NIEHS/UW Center for Child Environmental Health Risks Research, 1999-2015

Affiliate Investigator, Regional Primate Research Center, UW, 1995-2010

Core Faculty Member, Environmental Pathology Training Grant, Department of Pathology, University of Washington, 1980-present

Member, Graduate Faculty, University of Washington, 1979-present

Member, UW Human Subjects Policy Board, 2004-2008

Member, UW Tuition and Fees Policy Advisory Committee, 2006-2007

Member, Search Committee, School of Pharmacy Dean, 2007

Member, UW Data Policy Committee, 2006-2007

Member, Search Committee, Associated Vice Provost for Research-Compliance and Operations, 2006

Member, 5-year review of Dean, School of Medicine, UW, 2004-05

Member, Search Committee, Chair of Department of Biostatistics, School of Public Health, 2004-05

Member, Search Committee for Chair of Dept. Comparative Medicine, School of Medicine, UW

Chair, Search Committee, Director of the Public Health Sciences Division Laboratory, Fred Hutchinson Cancer Research Center, 2003.

Member, Search Committee for Proteomics Faculty position, School of Pharmacy, 2003.

Member, Council of Fellows, Pacific Northwest National Laboratories – UW (UW/PNNL) Joint Institutes, 2001-2004

Member, Faculty Advisory Committee for Office of Intellectual Property, 2001-2003

Member, Dept. Health Services Research Committee, 2001-2003

Member, Search Committee for Dental School Dean, 2000-02

Member, Dept. Environmental Health Curriculum & Teaching Policy Committee, 2000-2002

Member, *Ad Hoc* Task Force on Human Subjects, Office of the Provost, 2000-01

Editorial Board, Northwest Science and Technology, 1999-2000

Member, Provost's Review Committee for Dean of Pharmacy, 2000

Member, Search Committee, Molecular Epidemiology Faculty position, Fred Hutchinson Cancer Research Center, Public Health Sciences Division, 1999; 2001

Chair, Faculty Council, School of Public Health and Community Medicine, 1998-99

Member, Dean Search Committee, School of Public Health and Community Medicine, 1997-98

Curriculum Committee, Public Health Genetics Program, 1998-

Organizing Committee, interdisciplinary program on Public Health Genetics in the Context of Law, Ethics, and Policy 1997

Member, Friday Harbor Laboratory Advisory Board, University of Washington, 1994-1999

Co-Chairman for Exhibits, University of Washington Health Sciences Open House, 1991

Member, Search Committee for new Chairman of Department of Environmental Health, 1990-91

Member, Civil Engineering Program Review Committee, University Programmatic Review by the Graduate School, 1990

Member, Environmental Studies Review Committee, campus-wide review of Environmental Studies programs and future directions, for the Office of the Provost, 1990

Chairman, University of Washington Technical Oversight and Advisory Committee for Ruston/Vashon Arsenic contamination remedial investigation feasibility study; consultant to Black and Veatch and Washington Department of Ecology, 1987

Member, Search Committee for Occupational Medicine faculty position, 1987

Member, Appointments, Promotions and Tenure Committee, Department of Environmental Health, 1986-1992

Member, Arsenic Pathways Review committee, to assist study group and local health departments interpret and present findings of CDC/UW. Arsenic Pathways Study, 1986

Member, Alcohol and Drug Abuse Institute Executive Council, 1986-1989

Member, Small Grants Review Committee, Alcoholism and Drug Abuse Institute, University of Washington, 1984-86

Chair, Curriculum and Teaching Policy Committee, Institute for Environmental Studies, 1983

Member, Search Committee for Dean of School of Public Health and Community Medicine, University of Washington, 1982

Member, Curriculum and Teaching Policy committee, Department of Environmental Health, 1981-86; Chairman, 1985-86

Member, Search Committee for Director of Laboratories, Department of Environmental Health, 1980.

B. Invited Lectures

UW Law School – New Student Orientation – ‘Perspectives on Expert Witness Testimony’, Sept. 18, 2009.

Dept. Environmental Occupational Health Sciences Seminar, Toxicology and Risk Assessment of Dioxins – the NRC Report, Jan 18, 2007.

School of Public Health Distinguished Faculty Lecturer,, May 25, 2006.

UW Science Forum Speaker (University-wide Public Lecturer): Why me, Doc? What Scientists know – and don’t know, about the causes of cancer, April 18, 2006.

Panel Moderator and Discussant, “Research in the Future: UW in 2040”, a special symposium in honor of President Lee Huntsman, June 4, 2004.

Invited Speaker, Minority Undergraduate Program in Genome Sciences, Genes, Environment and Cancer, March 9, 2004.

Invited Speaker, Risk Communication: Going ‘right to know’ to ‘right to understand’., “Interpreting and communicating genetic risk information for susceptible populations”, April 3, 2003.

Course Director and Speaker, "Environmental Health for Reporters: Ethical and Policy Implications of the Human Genome Project and Genetic Research on Human Sensitivity to Environmental Pollutants", 6/1/2001; "Is it in Your Genes, or the Environment? Why Some People Develop Chronic Diseases and Others Don't", University of Washington.

Program Co-Organizer and Speaker, "Environmental Health For Educators", middle and high school teachers continuing education program, August 1997, August 1998, August 1999.

Invited Speaker, Summer Institute on Ecology & Environmental Sciences, National Science Teachers Association, "Genes, Environment and Cancer", 7/17/96;

Invites Speaker, Science Enhancement for Teachers Program, "Chemicals and Cancer", Biology Teaching Program, UW, May 16, 1996;

Seminar Speaker, "Glutathione S-transferases: Species and individual susceptibility to chemical carcinogens." Department of Medicinal Chemistry, UW, March 11, 1993.

Seminar Speaker, "Delaney Amendment of Carcinogenic Substances". The 1992 Symposium on Science, Technology, and Society, The Influence of Scientific Evidence on National Policy, April 28, 1992

Seminar Speaker, "Glutathione S-transferases: Species and individual susceptibility to chemical carcinogens." Department of Environmental Health, UW, February 5, 1988

Course Co-Organizer and Speaker, "Working With Pesticides: Health and Safety Issues", Northwest Center for Occupational Safety and Health, University of Washington, Seattle, WA, January 16, 1987

Seminar Speaker, "Role of Glutathione-S-Transferase in Detoxification of Aflatoxin B₁", Department of Environmental Health, University of Washington, Seattle, WA, December 4, 1986

Invited Speaker, "Basic Principles of Toxicology", given at "Worker Health and Safety at Hazardous Waste Sites", continuing Education Program, Department of Environmental Health, University of Washington, Seattle, WA, December 4, 1986

Panel Member and Discussant, "Scientist and the Media: Making Headlines on Your Own Terms", February 12, 1986; sponsored by University of Washington Medical Sciences Information Center

Speaker and Program Moderator, Professional exchange on hazardous/toxic substances in and around the home - use and disposal; sponsored by Institute for Environmental Studies, University of Washington, Seattle, WA, June 18, 1984

Invited Speaker, "Chemicals and cancer: Putting the risks in perspective"; Continuing Education Course, Biologists Look at Life and Human Affairs, Biology Program, University of Washington, Seattle, WA, April 16, 1984

Course Organizer and Instructor, "Legal aspects of toxicology"; Continuing Education Course, Educational Resource Center, Department of Environmental Health, University of Washington, SeaTac Hotel, Seattle, WA, December 2-3, 1983

Course Organizer and Instructor, "Principles and practice of toxicology in environmental health"; developed for Continuing Competency Education for Environmental Health Practitioners Program, University of Washington, Seattle, WA, January 25-March 15, 1982

Speaker, “Central nervous system effects of chemicals and physical agents”; Continuing Education Course, Educational Resource Center, University of Washington, Seattle, WA, April 20, 1981

Invited Speaker, “Heavy metals in the environment”; School of Public Health and Community Medicine's 10th Anniversary Celebration, University of Washington, Seattle, WA, September 25, 1980

Invited Speaker, “Occupational toxicology: principles of toxicology”; Department of Environmental Health Continuing Education Program, University of Washington, Seattle, WA, September 19, 1980

Seminar Speaker, “Carrier-mediated transport systems in isolated hepatocytes”; Department of Environmental Pharmacology, University of Washington, Seattle, WA, November 5, 1980

Seminar Speaker, “Effects of trace metals on metallothionein, glutathione, cytochrome P-450 and heme oxygenase activity in rats”, Department of Pathology, University of Washington. Seattle, WA, April 11, 1980.

12. PROFESSIONALLY-RELATED COMMUNITY SERVICE

Invited Speaker- Risk Assessment for Dioxins – NRC Panel Experience, US EPA/Wa State DOH, EPA Region 10 Offices, Seattle, Jan. 24, 2007, WA.

Invited Speaker – What Scientists Know- and don't Know- About the Causes of Cancer, Gilda's Club, Seattle, Jan 17, 2007.

Invited Speaker, Seattle Internal Medicine Group, ‘Genes, Environment and Cancer’, June 17, 2004.

Invited Speaker, “Implications of Genomic Information for Defining Susceptible Human Populations, USEPA, Region X, April 9, 2003.

Invited Speaker, “Risk Communication: going beyond ‘right to know’ to ‘right to understand’”, talk on Interpreting and Communicating genetic risk information for susceptible populations., Seattle, WA April 3, 2003.

Invited Speaker, Vashon Island Community Meeting, Health Risks from Arsenic in Soil, Vashon Island, WA, Nov. 18, 2002.

Invited Speaker, Washington State Board of Health, Genetics Working Group, “Basic Principles of Genomics in Public Health”, Jan. 3, 2002, Olympia, WA.

Course Organizer and Speaker, “Environmental and Occupational Health: A curriculum workshop for middle and high school educators”, Northwest Center for Occupational Safety and Health, UW, 7/19/96;

Seminar Speaker, “Small changes in enzyme structure can result in large changes in susceptibility to cancer”, Cancer Prevention Research Unit, Fred Hutchinson Cancer Research Center, 7/23/96;

Invited Speaker, Puget Sound Chapter, American Industrial Hygiene Assoc. “Genes, Environment and Cancer: Implications of the Human Genome Project”, 3/23/96

Invited Speaker, “Fundamentals of Toxicology”, Part 1 & Part 2, 1994 Northwest Federal Safety and Health Conference, Portland, OR, April 27, 1994

Co-organizer and Speaker: "Scientists in the Courtroom: The role of the expert witness", UW School of Public Health and Community Medicine and School of Law, December 9, 1993.

Instructor, Summer Institute in Toxicology, Northwest Center for Public Health Practice, Seattle, WA July 12-13, 1993

Invited Speaker, "Fundamentals of Toxicology", Part 1 & Part 2, 1992 Northwest Federal Safety and Health Conference, Olympia, WA, April 29, 1992

Invited Speaker, "Basic Principles of Risk Assessment", American Public Works Association, Washington State Chapter, Spring Conference, March 26, 1992

Invited Speaker, "Toxic Chemicals, Human Health and the Environment", Science Enhancement for Teachers Program, University of Washington, April 27, 1991

Invited Speaker, "Are rodents good models for human responses to cancer-causing chemicals? Aflatoxin as an example. WEST '91 Conference, Seattle, Convention Center, April 11, 1991

Invited Speaker, "America's Epidemic of Chemicals and Cancer - Myth or Fact?" Inland Empire Agricultural Chemical Association 18th annual convention, Spokane, WA Dec. 11, 1990

Invited Panel Participant, Land Based Marine Pollution in the Pacific Northwest, sponsored by the Ocean Studies Council, University of British Columbia, Vancouver, BC, Nov. 2, 1991

Program Organizer and Speaker, "Human Health Risk Assessment: Process and Limitations," principal lecture for a symposium on uncertainties in risk assessment and risk management, given to upper level managers in the Washington Department of Ecology, March 13, 1990

Invited Speaker, "Risk Assessment in Foods", and Panel participant, "Chemical Residues in Foods", presented at Food Safety in Northwest Supermarkets, sponsored by Washington State University Cooperative Extension, Culinary Botany Northwest, and Department of Environmental Health, UW. October 12, 1989

Invited Guest, "Pesticides in Our Food", The Jim Altoff Show, KING 1090 Radio, October 9, 1989 (1 hour)

Invited Speaker, "Pesticide residues in our food and water- a hazard to your health, or a negligible risk?" Saturday Seminar Series, sponsored by the Office of University Relations and UW Extension, Sept. 30, 1989

Seminar Speaker, "Biochemical basis for Species Differences in Aflatoxin carcinogenesis", Washington State University Pharmacology/Toxicology Program Seminar, July 25, 1989

Invited Participant, "Town Meeting", local television talk show focusing on health controversies over pesticide residues in food, KOMO TV, Seattle, WA, April 13, 1989.

Invited Speaker, "Strengths and Weaknesses of Toxicological Evidence", presented at Continuing Education Program on "Legal Aspects of Occupational Health", University of Washington Education Resource Center, February 8, 1989, Seattle, WA.

Co-organizer and Speaker, "Pesticides in the Urban Environment: Health and Environmental Responsibility", program for 1988 annual convention of the International Pesticide Applicators Association, held in Bellevue, WA on Sept. 28-30, 1988

Invited Speaker, City Club (a downtown business leaders civic group). Discussion of the procedures and difficulties encountered in utilizing science in decision making regarding public health risks. Seattle, WA, February 23, 1988

Invited Speaker, "Utilization of Scientific Information in Risk Management Decisions", presented to Division Managers and Budget Coordinators for METRO, Battelle Conference Center, Seattle, WA, October 14, 1987

Invited Speaker, "Problems in Risk assessment and Risk Communication", King County Health Department annual retreat, Seattle Center, Seattle, WA, March 18, 1987

Invited Speaker, "Taking Care of Yourself - Pesticide Use and Human Health", presented at 77th Annual Meeting of the Western Washington Horticultural Association, Olympia, WA, January 6, 1987

Invited Speaker, "Toxic Chemicals and Public Health: Putting the Risks in Perspective", presented to the Seattle Chapter of the American Society of Safety Engineers, October 27, 1986

Invited Speaker, "Principles of Toxicology", given at "Toxic Substances and Public Health Workshop", sponsored by the Indian Health Service, Seattle, WA, October 22, 1986

Invited Speaker, "Pesticides and Public Health: Risks and Uncertainties", presented at the International Pesticide Applicators Association Convention, Spokane, WA, October 2, 1986

Invited Speaker, "Basic Principles of Toxicology", Risk Assessment Workshop for State Legislators Olympia, WA. Sponsored by Washington Department of Social and Health Services, September 25, 1986

Invited Speaker, "Basic Principles of Toxicology and Risk Assessment", presented to Community Health nurses and doctors for the Seattle-King County Health Department, Seattle, WA, June 10, 1986

Invited Speaker, "Potential health effects of occupational Exposure to PCB's", presented to Snohomish County PUD utility workers, March 17, 1986

Invited Speaker, "Health Implications of Gaseous Emissions Associated with Midway Landfill", February 6, 1986, Kent, WA; sponsored by Washington State Department of Ecology

Course Co-Organizer and Instructor, "Basic Principles of Toxicology for Environmental/ Health Practitioners"; two day course given in Tacoma, WA (July 16 & 17, 1985), and Spokane, WA (August 29 & 30, 1985)

Speaker, "Toxicology of PBC's, dioxins and dibenzofurans", given at a public meeting in Kitsap County at the request of Seattle City Light Environmental Affairs Division, Seattle, WA, June 17, 1985

Invited Speaker, "An evaluation of the health concerns over dioxins found in Eagle Harbor, WA", given at a community meeting at the request of Dr. Willa Fisher, Director of the Kitsap County Health Department, July 11, 1985

Invited Speaker, "Health Impacts of Environmental Pollutants", given for the Continuing Medical Education Series, Riverton Hospital, July 19, 1985

Invited Speaker, "Health Effects of PCB's", given to Seattle City Light employees on November 1, 1984, January 9, November 19, November 21, December 4, and December 11, 1985

Course Organizer and Speaker, "Toxic Chemicals: Communicating Risks to the Public", forum for Scientist-media exchange, sponsored by the Society of Toxicology and the Department of Environmental Health, Battelle-Seattle Conference Center, Seattle, WA, April 27, 1985

Invited Speaker, Public Perception of Health Impacts of Chronic Pesticide Exposure; International Pesticide Applicators Association Convention, Fife, WA, September 27, 1984

Course Organizer and Instructor, Toxicology Review Course for EPA Personnel; Seattle, WA, September 5, 1984-January 15, 1985 (attended by 45 EPA staff)

Invited Speaker, "Pesticides use for Gypsy Moth control", presented at Controversies in Poisoning Management and Occupational/Environmental Exposures, Children's Hospital and Medical Center, Seattle, WA, April 28-29, 1983

Lecturer, "Recent Developments in Biological Monitoring"; Continuing Medical Education Course, Seattle, WA, March 18-19, 1983

Lecturer, Toxicology; short course presented to Hazards Assessment Program employees of NOAA, 3 lectures; NOAA Sand Point Facilities, Seattle, WA, March 10, 1983

Speaker, Health implications of Gypsy Moth control programs; Public Information Seminar, sponsored by Department of Environmental Health and Institute for Environmental Studies, University of Washington, March 10, 1983

Invited Speaker, "Health effects of chronic pesticide exposure: a training program of health personnel: prevention, recognition and treatment of pesticide-related illness"; sponsored by Yakima Valley Farm Workers Clinic and the Migrant Health Clinics in Washington, Oregon and Idaho, Pasco, WA, January 21-22, 1983

Invited Speaker, "Clinical toxicology of pesticides"; Northwest Poison Control Center Conference, Spokane, WA, April 22-23, 1982

Invited Speaker, "A survey of toxic torts"; Washington State Trial Lawyers Association Annual Meeting, Vancouver, BC, Canada, July 10, 1981

Invited Speaker, "Carcinogens in the environment"; Washington State Office of Environmental Education in Health Education, Everett, WA, March 10, 1981

Invited Speaker, "Phenoxy acid herbicides--fact and fiction"; Huxley College of Environmental Studies, Western Washington University. Bellingham, WA, January 27, 1981

Invited Speaker, "Central nervous system effects of industrial chemicals and physical agents"; 1980 Occupational Health and Medical Conference, Spokane, WA, October 22-24, 1980

Invited Speaker, "PCB's and human health". Washington State Office of Environmental Education and Health Education. Seattle, WA, February 27, 1980.

13. TEACHING HISTORY

A. Recent Teaching (2011-2018):

I co-taught, with Dr. Thummel (Chair, Dept. Pharmaceutics) PHG/ ENVH/PCEUT 513, Fundamentals of Pharmacogenetics and Toxicogenomics. Enrollment is typically 20-25 graduate students. I had 50% responsibility for this course.

I provided 5 lectures each year to the three quarter graduate Toxicology series, ENV514 (1), ENVH 515 (3) and ENVH 516 (1).

B. Previous Teaching:

In 2012 I adapted a previous ENVH 590A graduate toxicology course to a new format. This was a 4 credit course, with enrollment of 15-20 non-toxicology graduate students. The course utilized the lecture content of ENVH 405, of which I give 50% of the lectures, plus included a 1 hr per week discussion group with the graduate students. I was responsible for assigning the readings for each weekly session, and helping the students who lead the discussion. I pass this course on to others when I became Dean of the Graduate School.

From 1979 - ~1985, I developed and taught a 1 quarter in depth Toxicology course, ENVH 515, with enrollment of ~10- 15 students. I also co-taught ENVS 101, Introduction to Environmental Studies (5 credits 200-300 students) at least once a year from 1979-1983.

From ~1986-1995 I had primary responsibility for teaching one of the three quarter toxicology courses.

From ~ 1990 – 2000 (when I devoted 40% effort as Associate Dean for Research in the School of Public Health, and then the 40% Position as Associate Vice Provost for Research), I had 100% responsibility for ENVH 405, with typically 30-50 students enrolled. I also had either 50% or 100% responsibility for ENVH 567 – Environmental Carcinogenesis (3 credits, typical enrollment of 6-12 students).

I Taught one Lecture to Public Health Genetics 200 in fall and spring quarters, and undergraduate course with about 80 students for 5 years. The course was led by Dr. Patricia Kuzsler in the Law School.

In 2010 I developed, with Dan Luchtel, a graduate level course in toxicology for non-toxicology EHS doctoral students in occupational medicine and exposure sciences (and other programs). I was 50% responsible for this in 2010 and 2011. The course was restricted in 2012, as discussed above, and I still maintain 50% responsibility for it.

C. Graduate Student Mentoring:

MS/MSPH Students (Preceptor)

Brian Toal	Joel Rank	Misha Trusty
Leslie Carpenter	Denise Hamel	Julie Hill
Margaret Stinson	Karyn Micheleson	Jason VanLoo
Heidi Hagelstein	Ingrid Borroz	Marc Stifleman
Julia Richards	Dana Stahl	Kate Bradley

PhD. Students Mentored

Trainee Name	Training Period	Degree, Date, Institution	Project Title	Current Position
Carolyn Holeski	83-86	BS, UC-Davis, Toxicology; PhD, UW Pathology, 1986	Effects of enzyme inducers on the hepatobiliary disposition of Aflatoxin B1 in the rat	MD in private practice
David H. Monroe	84-87	UW, Ph.D., 12/87	Species and Diet Related Resistance to Chemical Carcinogens: Aflatoxin B1	Staff Scientist, USEPA, Region VI, Kansas City, MO
Van Ness, Kirk	92-95	MS, 1986, University of Washington	Identification of amino acids in glutathione S-transferase mYc responsible for high activity toward aflatoxin oxide	Research Scientist, Zymogenetics, Seattle, WA (recently laid off)
Wang, Charles (Changhong)	94-99	MSPH, 1988; MD, Tongji Medical School	Complementary DNA cloning and sequence of alpha class GSTs in monkey liver	Associate Professor Dept. Medicine/CSMC, UCLA David Geffen School of Medicine, Los Angeles, CA
*McHugh, Tom	94-98	MS, 1993, Stanford University	Molecular characterization of glutathione transferase with high activity toward aflatoxin 8,9epoxide	Toxicologist, Groundwater Services International, Houston, TX
*Smith, Helen	98-2004	BS, U. Texas-Austin, Pharmacy, 1989; MS, Community Health, UT-Houston, 1994; Ph.D. UW, 2004	Polymorphisms in estradiol metabolism in human endometrium	Associate Professor, School of Pharmacy, University of the Incarnate Word, San Antonio, TX
Abel, Erica	99-2004	BS, Texas A & M, 1997; Ph.D. UW, 2004	Structure-function studies on glutathione S-transferases	Research Assistant Professor, UT-MD Anderson Cancer Center, Carcinogenesis Program, Smithville, TX
Guo, Yingying	99-2004	MD, Sun Yat-sen University, Gungzhou Province, China, 1994; MS, Univ. Cincinnati, 1998; Ph.D. UW, 2004	Effects of aflatoxin epoxide mediated DNA damage on global gene expression in yeast and HepG2 cells and human hepatocytes.	Research Scientist, Eli Lilly & Co, Indianapolis, IN

Trainee Name	Training Period	Degree, Date, Institution	Project Title	Current Position
Poulton, Emma-Jane	2004-2010	B.S., Chemistry, 2004, MIT	The phytochemical, sulforaphane is a sensitive and specific inhibitor of the human steroid X-receptor (SXR)	Research Scientist, Sanofi-Aventis Corp. Bridgewater, NJ
Peck, Erin	204-2010	B.S., Chemistry 2002, Univ. North Carolina, Chapel Hill	Role of human CYP1A2 in the activation and detoxification of aristolochic acid	Resident, Dept. Family Medicine and Community Health, University of Wisconsin School of Medicine
Vandivort, Tyler (co-mentor with Bill Parks, Pulmonary Medicine)	2011-15		The Function and Regulation of Macrophage Matrix Metalloproteinase 10 (MMP10) in Lung Injury and Fibrosis	Research Scientist, Charles River, Inc. Las Vegas, NV
Cook, Travis (co-mentor with Jing Zhang, Pathology)	2011-2014		Mechanisms of Mn-induced Parkinson's disease	Toxicologist, Gradient Corp. Seattle, WA
Shi-Yu (Shirley) Chang	2012-2016		Development of a microphysiological system using human liver cells	Post-doctoral fellow, School of Pharmacy, Univ. WA, Seattle, WA

Post-doctoral Fellows Mentored:

Trainee Name	Training Period	Previous Degree, Date, Institution	Project Title	Current Position
*Ramsdell, Howard	87-89	Ph.D., 1989, Oregon State University	Biochemical basis for species differences in aflatoxin biotransformation	Assoc. Professor, Dept. Environ. Hlth, Colo. State Univ., Fort Collins, CO
Buetler, Timo	90-94	Ph.D., 1989, University of Basel, Switzerland	Molecular cloning and biochemical characterization of alpha class glutathione transferase in mouse liver	Senior Scientist, Nestle, Inc., Basal, Switzerland
Chen, Zhi-Ying	90-95	1970, M.D., Sun Yat Sen University	Effects of enzyme inducers on liver preneoplastic lesions	Research Scientist, UCLA School of Medicine

Trainee Name	Training Period	Previous Degree, Date, Institution	Project Title	Current Position
*Gallagher, Evan	91-94	Ph.D., 1991, Duke	Kinetics of CYP1A2 and 3A4-mediated aflatoxin activation and detoxification	Professor, Environ. Occup. Health Sciences, UW, Seattle, WA
Bammler, Theo	95-2000	Ph.D., University of Dundee, Scotland	Effects of Oltipraz and ethoxyquin on disposition of aflatoxin B1 in the marmoset monkey	Res. Scientist, Manager of Microarray Facility, Center for Ecogenetics, UW, Seattle, WA
Kelly, Edward	96-2001	Ph.D., 1996, Univ. Washington	Role of human epoxide hydrolase in the detoxification of aflatoxin B1	Research Scientist, Dept. Pharmaceuticals, UW, Seattle, WA
Gross-Steinmeyer	2002 - 06	PhD,	Phytochemical perturbations of aflatoxin biotransformation	Currently 'at home' mother
Smith, Wesley	2008-2012	PhD, Univ. Montana	Toxicology of QDot nanomaterials	Risk Assessor, California State Office of Environmental Health Hazards Assessment

I currently serve as a member of the Doctoral Committees of Megan Cartwright and David Scoville, both in the Toxicology PhD program in DEOHS.

I have also served on many Doctoral Dissertation and Reading committees for PhD students in Environmental Health, Epidemiology, Pharmaceuticals, Medicinal Chemistry, and Pharmacology.

B. Dr. Eaton's prior testimony, 2014-2019

City of Clovis v. Shell Oil Company dba Shell Chemical Company *et al.*, 2016
Los Angeles Superior Court Case No. SC085170
Trial testimony on behalf of defendants, November 28, 2016

ROSLYN DAUBER AND JOHN DI COSTANZO, PLAINTIFFS,
VS. MONSANTO COMPANY, ET AL., DEFENDANTS. CASE NO. BC483342
SUPERIOR COURT OF THE STATE OF CALIFORNIA CASE NO. BC 49758
Deposition, Feb. 16, 2016; Trial testimony on behalf of defendants, March 23, 2016

PAUL BROWNLIE, FRED STEELE and ARUTYUN KARABADZHAKYAN, PLAINTIFFS, vs. MONSANTO COMPANY, ET AL., DEFENDANTS.
SUPERIOR COURT OF THE STATE OF CALIFORNIA CASE NO. BC 49758
Trial testimony on behalf of defendants April 23, 2016

BENITO WALKER, ET AL., Plaintiffs, vs. MONSANTO COMPANY, ET AL., Defendants.
CIRCUIT COURT OF THE CITY OF ST. LOUIS, MO. Case # 1122-CC09621-01
Trial testimony on behalf of defendants, May 23, 2016

City of Atwater v. Shell Oil Company, Superior Court of California, County of San Francisco, Case No. CGC 05 441058
Trial testimony, August 5, 2019, Fresno, CA.

SAN DIEGO UNIFIED PORT DISTRICT ET AL, Plaintiffs, vs. MONSANTO COMPANY, ET AL., Defendants.
UNITED STATES DISTRICT COURT FOR THE SOUTHERN DISTRICT OF CALIFORNIA, CASE NO. 3:15-CV-00578-WQH-AGS
Deposition on behalf of defendants, June 9, 2019

VI. Appendix 2: Exposure assessment

A. PCB daily intake rate estimates for congeners measured in the Spokane River

Daily congener-specific fish intake rates for all congeners measured and detected in fish samples from the Spokane River were derived as discussed in the main body of the report (Section II.A). Of the derivations presented in Section II.A., methods I and III represent the best and reasonable upper bounds for daily intake from Spokane River fish consumption, respectively. Table A2-1 provides congener-specific mean daily intake.

Table A2-1. Intake rates of PCB congeners measured in fish from the Spokane River

Congener	Method I	Method III
	Mean FCR, mean percentile tissue (ng/d) [Best estimate]	95 th FCR/mean tissue (ng/d) [Reasonable upper bounds]
PCB1	0.00101	0.00435
PCB2	0.00079	0.00313
PCB3	0.00151	0.00631
PCB4	0.00782	0.03352
PCB5	8.00E-05	3.00E-04
PCB6	0.00538	0.02238
PCB7	0.00069	0.0028
PCB8	0.05827	0.2923
PCB9	0.00135	0.0056
PCB10	0.00032	0.00138
PCB11	0.05449	0.19608
PCB12/13	0.00121	0.00418
PCB14	0	0
PCB15	0.01272	0.045
PCB16	0.10917	0.51439
PCB17	0.21409	0.99259
PCB18/30	0.31921	1.45253
PCB19	0.01916	0.08165
PCB20/28	2.39257	10.58827
PCB21/33	0.2929	1.28272
PCB22	0.3334	1.45152
PCB23	0.00046	0.00198
PCB24	0.0039	0.01698
PCB25	0.10736	0.46696
PCB26/29	0.31766	1.4423
PCB27	0.03126	0.13499
PCB31	1.29944	5.84945
PCB32	0.14184	0.66663

Congener	Method I	Method III
	Mean FCR, mean percentile	95 th FCR/mean tissue (ng/d)
	tissue (ng/d)	
	[Best estimate]	[Reasonable upper bounds]
PCB34	0.01071	0.04596
PCB35	7.00E-05	0
PCB36	0.00138	0.00758
PCB37	0.12143	0.54447
PCB38	0.00771	0.03344
PCB39	0.00685	0.0363
PCB40/41/71	1.8762	8.199
PCB42	1.43168	6.26495
PCB43	0.15846	0.68416
PCB44/47/65	5.57461	24.62993
PCB45/51	0.39088	1.75981
PCB46	0.06471	0.27723
PCB48	0.89349	3.9822
PCB49/69	4.29878	19.09404
PCB50/53	0.34144	1.53198
PCB52	6.62485	29.82538
PCB54	0.00105	0.00437
PCB55	0.00785	0.02658
PCB56	1.93465	8.34638
PCB57	0.02893	0.12481
PCB58	0.03332	0.14941
PCB59/62/75	0.50988	2.24317
PCB60	2.32807	10.22005
PCB61/70/74/76	13.29028	58.88191
PCB63	0.57192	2.50392
PCB64	3.01743	13.32164
PCB66	10.35575	45.55692
PCB67	0.14103	0.60842
PCB68	0.06047	0.25825
PCB72	0.08761	0.37628
PCB73	0.00257	0.01018
PCB77	0.37176	1.64768
PCB78	0	0
PCB79	0.15233	0.66827
PCB80	0	0
PCB81	0.03234	0.14036
PCB82	1.1911	5.12407
PCB83	0.34482	1.93057

Congener	Method I	Method III
	Mean FCR, mean percentile	95 th FCR/mean tissue (ng/d)
	tissue (ng/d)	
	[Best estimate]	[Reasonable upper bounds]
PCB83/99	13.93208	64.80253
PCB84	1.24137	5.42217
PCB85/110/115/116/117	9.34416	47.55615
PCB85/116/117	4.07479	18.13212
PCB86/087/097/108/119/125	8.09797	36.05392
PCB86/087/097/109/119/125	2.99682	14.69859
PCB88/091	1.58789	7.04818
PCB89	0.00743	0.02938
PCB90/101/113	13.71458	62.17174
PCB92	2.35211	10.65798
PCB93/95/98/100/102	3.88144	16.47932
PCB93/098/100/102	0.07431	0.33906
PCB94	0.02466	0.1044
PCB95	1.3959	6.95928
PCB96	0.03042	0.13054
PCB99	1.48529	8.31591
PCB103	0.07067	0.30514
PCB104	0.00028	0.00112
PCB105	6.6143	29.82731
PCB106	0.00405	0.01111
PCB107	0.78492	4.01892
PCB107/124	0.42903	1.94896
PCB108/124	0.2556	1.31032
PCB109	1.3174	5.8432
PCB110/115	11.6817	53.17627
PCB111	0.01194	0.05358
PCB112	0	0
PCB114	0.54026	2.40755
PCB118	17.39323	78.28041
PCB120	0.05503	0.23394
PCB121	0.00059	0.00211
PCB122	0.11343	0.48433
PCB123	0.40332	1.8151
PCB126	0.04591	0.21727
PCB127	0.00787	0.03491
PCB128/166	2.63087	12.02337
PCB129/138/160/163	11.21993	46.5088
PCB129/138/163	7.64328	35.44161

Congener	Method I	Method III
	Mean FCR, mean percentile	95 th FCR/mean tissue (ng/d)
	tissue (ng/d)	
	[Best estimate]	[Reasonable upper bounds]
PCB130	0.97077	4.37431
PCB131	0.09327	0.38418
PCB132	2.03889	8.81592
PCB133	0.26455	1.18185
PCB134/143	0.34147	1.5059
PCB135/151	1.09083	5.17741
PCB135/151/154	2.23891	9.41729
PCB136	0.49287	2.1853
PCB137	0.87723	3.97257
PCB137/164	1.78417	9.32505
PCB139/140	0.30376	1.36867
PCB141	2.44813	11.14256
PCB142	0	0
PCB144	0.46728	2.08639
PCB145	0.00164	0.00738
PCB146	2.93675	13.2368
PCB147/149	6.05371	27.44543
PCB148	0.01604	0.06807
PCB150	0.00867	0.03625
PCB152	0.00422	0.01751
PCB153/168	15.97166	72.12338
PCB154	0.04009	0.17032
PCB155	0.00665	0.02137
PCB156/157	1.98674	9.0544
PCB158	1.63018	7.4697
PCB159	0.0681	0.28874
PCB160	0.00305	0.00449
PCB161	0	0
PCB162	0.07345	0.33833
PCB164	0.63589	2.9687
PCB165	0.00504	0.02132
PCB167	0.75096	3.39874
PCB169	0.00127	0.00418
PCB170	2.97956	13.44763
PCB171/173	0.87299	3.907
PCB172	0.68477	3.05006
PCB174	1.65283	7.29709
PCB175	0.14428	0.63441

Congener	Method I	Method III
	Mean FCR, mean percentile	95 th FCR/mean tissue (ng/d)
	tissue (ng/d)	
	[Best estimate]	[Reasonable upper bounds]
PCB176	0.15296	0.64749
PCB177	1.50875	6.73234
PCB178	0.73568	3.27093
PCB179	0.51081	2.25545
PCB180/193	8.75396	38.52057
PCB181	0.02872	0.12343
PCB182	0.02534	0.10703
PCB183	1.18255	5.69294
PCB183/185	2.40676	10.7223
PCB184	0.01416	0.05193
PCB185	0.07845	0.34299
PCB186	0	0
PCB187	5.99809	26.55919
PCB188	0.01039	0.04375
PCB189	0.10085	0.4384
PCB190	0.60392	2.68968
PCB191	0.12075	0.53097
PCB192	0.00162	0.00222
PCB194	1.45435	6.30157
PCB195	0.4862	2.1581
PCB196	0.96629	4.30965
PCB197	0.02718	0.13098
PCB197/200	0.11517	0.46826
PCB198/199	2.58681	11.38372
PCB200	0.01336	0.04497
PCB201	0.21835	0.9603
PCB202	0.40692	1.7576
PCB203	1.73995	7.71464
PCB204	0.00056	0.00203
PCB205	0.05773	0.24251
PCB206	0.84613	3.63197
PCB207	0.10503	0.44184
PCB208	0.21599	0.92562
PCB209	0.11059	0.47525

VII. Appendix 3: Cancer and mode of action for PCBs

A. Mutagenicity of PCB mixtures and specific congeners

Over the past 50 years, there have been approximately 275 different mutagenesis assays on PCB mixtures (Aroclors, Kanaclors, Clophens, simulated mixtures, individual congeners, and specific PCB metabolites). There are at least a dozen different types of assays that provide some measure of DNA damage or mutagenicity. Table A3-1 and Table A3-2 show a combined 100 different mutagenicity/DNA damage assays that have been performed on just Aroclors 1242, 1254 and 1260. Out of these 100 different assays, 42 used *in vitro* tests and 58 used *in vivo* exposures. Of the 42 *in vitro* tests, 35 were negative or inconclusive, demonstrating the remarkable lack of mutagenic potential of Aroclors. Of the 58 *in vivo* tests, 12 were positive. Four of these 12 studies were chromosomal aberrations seen in fish cells administered large (50 – 300 mg/kg) doses of Aroclor 1254. These 4 studies provide no human-relevant information. Three of the positive *in vivo* studies examined 8-OHdG adducts, a measure of oxidative stress rather than direct DNA damage to DNA.

Many additional studies have been done on specific PCB congeners, the vast majority of which were negative. Overall, they provide relatively little useful information about the mutagenic potential to humans of PCBs found in fish. Some of these studies used 3-ChloroPCB, and the hydroxylated metabolite of 3-Chloro PCB, and are irrelevant to the assessment of PCBs in the environment since these compounds are found only at very low levels (<0.001% of total) amounts in fish.

Looking at the total body of evidence relating to whether mutagenesis by PCBs found in fish could be a significant contributor to the carcinogenic effects seen in some 2 year bioassays with Aroclors, it is my opinion that mutagenesis/DNA damage plays no significant role in the development of tumors seen in laboratory animals used by the US EPA and the Washington Department of Health in various human health risk assessments of PCBs found in the environment. This conclusion was also reached in a detailed review of the putative modes of action of PCBs in carcinogenesis published by others (Andersen *et al.*, 2013; Glauert *et al.*, 2008; Knerr and Schrenk, 2006).

Table A3-1. *In vitro* mutagenesis studies with Aroclors 1242, 1254, and 1260

Assay	Species (strain or cell type)	Study design	Aroclor 1242*	Aroclor 1254*	Aroclor 1260*	Reference
Chromosomal aberrations	Human (Peripheral lymphocytes)	48-hr incubation		+ (0.01 µg/mL)		(Sargent <i>et al.</i> , 1989)
DNA adducts	Human (Primary hepatocytes)	0-230 µM for 24, 48, or 96 hrs (depending on donor)		(+) weakly positive, 20 µg/mL (60 µM)		(Borlak <i>et al.</i> , 2003)
DNA adducts, 32P-postlabelling	Human (HepG2)	50 µM added 24h after plating		- (50 µM or 17 µg/mL)		(Dubois <i>et al.</i> , 1995)
DNA adducts, 32P-postlabelling	Rat (Primary liver cells)	50 µM added 24h after plating		- (50 µM or 17 µg/mL)		(Dubois <i>et al.</i> , 1995)

Assay	Species (strain or cell type)	Study design	Aroclor 1242*	Aroclor 1254*	Aroclor 1260*	Reference
DNA adducts, detection of repairable adducts by growth inhibition (DRAG)	Hamster (Wild type (AA8) and DNA repair-deficient Chinese hamster ovary cells (EM9, UV4, UV5))	Cells treated for 24-hr		- (135; 114, 127, 132 µg/mL IC50's for AA8, EM9, UV4, UV5 cells)		(Johansson <i>et al.</i> , 2004)
DNA integrity (acridine orange staining)	Rat (Sperm)	3h incubation (0.01, 0.1, 1 µM)		+ (0.003 µg/mL or 0.01 µM)		(Aly, 2013)
DNA strand breaks (comet assay)	Rat (Primary prostate cells)	Cells exposed to 1 µg/mL for 24 hr		+ (1 µg/mL)		(Cillo <i>et al.</i> , 2007)
DNA strand breaks (single-strand breaks; alkaline elution)	Rat (Primary hepatocytes)	3 hr exposure		+ (100 µg/mL or 0.3 mM)		(Sina <i>et al.</i> , 1983)
Gene mutation	Hamster (Chinese V79 lung)	Incubate with 50, 100, or 150 µg/mL	- (150 µg/mL)			(Hattula, 1985)
Gene mutation	Hamster (Syrian Hamster Embryo Cells)	Up to 50 µg/mL		- (50 µg/mL)		(Pienta, 1980)
Micronucleus formation	Human (Keratinocytes)	Cells exposed for 3 days to 10 µM		- (3 µg/mL or 10 µM)		(van Pelt <i>et al.</i> , 1991)
Non-mammalian systems, Ames Assay, reverse mutation	Escherichia coli (WP-2 and WP-2 uvrA-)	conc. not given (with S9 activation)		- (conc. not given)		(Probst <i>et al.</i> , 1981)
Non-mammalian systems, Ames Assay, reverse mutation	Escherichia coli (WP-2 and WP-2 uvrA-)	conc. not given (without S9 activation)		- (conc. not given)		(Probst <i>et al.</i> , 1981)
Non-mammalian systems, Ames Assay, reverse mutation	Escherichia coli (WP-2 uvrA)	0.3 to 333.3 µg/plate (without S9 activation)		- (333 µg/plate)		(Dunkel <i>et al.</i> , 1984)
Non-mammalian systems, Ames Assay, reverse mutation	Escherichia coli (WP-2 uvrA)	0.3 to 333.3 µg/plate (with S9 activation)		- (333 µg/plate)		(Dunkel <i>et al.</i> , 1984)
Non-mammalian systems, Ames Assay, reverse mutation	Escherichia coli (WP-2 uvrA)	up to 177 µg/plate (without S9 activation)		- (177 µg/plate)		(Evandri <i>et al.</i> , 2003)
Non-mammalian systems, Ames Assay, reverse mutation	Escherichia coli (WP-2 uvrA)	up to 177 µg/plate (with S9 activation)		- (177 µg/plate)		(Evandri <i>et al.</i> , 2003)
Non-mammalian systems, Ames Assay, reverse mutation	Salmonella typhimurium (G46, C3076, D3052)	conc. not given (with S9 activation)		- (conc. not given)		(Probst <i>et al.</i> , 1981)

Assay	Species (strain or cell type)	Study design	Aroclor 1242*	Aroclor 1254*	Aroclor 1260*	Reference
Non-mammalian systems, Ames Assay, reverse mutation	Salmonella typhimurium (G46, C3076, D3052)	conc. not given (without S9 activation)		- (conc. not given)		(Probst <i>et al.</i> , 1981)
Non-mammalian systems, Ames Assay, reverse mutation	Salmonella typhimurium (TA1535, TA1537)	to 500 µg/plate (without S9 activation)		- (500 µg/plate)		(Schoeny <i>et al.</i> , 1979)
Non-mammalian systems, Ames Assay, reverse mutation	Salmonella typhimurium (TA1535, TA1537)	to 500 µg/plate (with S9 activation)		- (500 µg/plate)		(Schoeny <i>et al.</i> , 1979)
Non-mammalian systems, Ames Assay, reverse mutation	Salmonella typhimurium (TA1535, TA1537, TA1538)	conc. not given (with S9 activation)		- (conc. not given)		(Probst <i>et al.</i> , 1981)
Non-mammalian systems, Ames Assay, reverse mutation	Salmonella typhimurium (TA1535, TA1537, TA1538)	conc. not given (without S9 activation)		- (conc. not given)		(Probst <i>et al.</i> , 1981)
Non-mammalian systems, Ames Assay, reverse mutation	Salmonella typhimurium (TA1535, TA1537, TA1538)	0.3 to 333.3 µg/plate (without S9 activation)		- (333 µg/plate)		(Dunkel <i>et al.</i> , 1984)
Non-mammalian systems, Ames Assay, reverse mutation	Salmonella typhimurium (TA1535, TA1537, TA1538)	0.3 to 333.3 µg/plate (with S9 activation)		- (333 µg/plate)		(Dunkel <i>et al.</i> , 1984)
Non-mammalian systems, Ames Assay, reverse mutation	Salmonella typhimurium (TA98, TA100)	0.05, 0.5, 5, 50, or 500 µg/plate (without metabolic activation)		- (500 µg/plate)		(Bruce and Heddle, 1979)
Non-mammalian systems, Ames Assay, reverse mutation	Salmonella typhimurium (TA98, TA100)	0.05, 0.5, 5, 50, or 500 µg/plate (with S9 metabolic activation)		- (500 µg/plate)		(Bruce and Heddle, 1979)
Non-mammalian systems, Ames Assay, reverse mutation	Salmonella typhimurium (TA98, TA100)	to 500 µg/plate (without S9 activation)		- (500 µg/plate)		(Schoeny <i>et al.</i> , 1979)
Non-mammalian systems, Ames Assay, reverse mutation	Salmonella typhimurium (TA98, TA100)	to 500 µg/plate (with S9 activation)		- (500 µg/plate)		(Schoeny <i>et al.</i> , 1979)
Non-mammalian systems, Ames Assay, reverse mutation	Salmonella typhimurium (TA98, TA100)	0.3 to 333.3 µg/plate (without S9 activation)		- (333 µg/plate)		(Dunkel <i>et al.</i> , 1984)
Non-mammalian systems, Ames Assay, reverse mutation	Salmonella typhimurium (TA98, TA100)	0.3 to 333.3 µg/plate (with S9 activation)		- (333 µg/plate)		(Dunkel <i>et al.</i> , 1984)

Assay	Species (strain or cell type)	Study design	Aroclor 1242*	Aroclor 1254*	Aroclor 1260*	Reference
Non-mammalian systems, Ames Assay, reverse mutation	Salmonella typhimurium (TA98, TA100)	up to 177 µg/plate (without S9 activation)		- (177 µg/plate)		(Evandri <i>et al.</i> , 2003)
Non-mammalian systems, Ames Assay, reverse mutation	Salmonella typhimurium (TA98, TA100)	up to 177 µg/plate (with S9 activation)		- (177 µg/plate)		(Evandri <i>et al.</i> , 2003)
Non-mammalian systems, Ames Assay, reverse mutation	Salmonella typhimurium (TA98, TA1000)	conc. not given (with S9 activation)		- (conc. not given)		(Probst <i>et al.</i> , 1981)
Non-mammalian systems, Ames Assay, reverse mutation	Salmonella typhimurium (TA98, TA1000)	conc. not given (without S9 activation)		- (conc. not given)		(Probst <i>et al.</i> , 1981)
Non-mammalian systems, Ames Assay, reverse mutation	Salmonella typhimurium (TA98, TA1538)	50-5,000 µg/plate (without S9 activation)		- (5,000 µg/plate)		(Shahin <i>et al.</i> , 1979)
Non-mammalian systems, Ames Assay, reverse mutation	Salmonella typhimurium (TA98, TA1538)	50-5,000 µg/plate (with S9 activation)		- (5,000 µg/plate)		(Shahin <i>et al.</i> , 1979)
Non-mammalian systems, Chromosomal aberrations	Allium cepa L. (Onion)	0, 0.3, 3, 8, or 30 µg/mL		+ (8 µg/mL)		(Evandri <i>et al.</i> , 2003)
Non-mammalian systems, DNA adducts, 32P-postlabelling	Quail (Primary egg cells)	50 µM added 24 hr after plating		- (50 µM or 17 µg/mL)		(Dubois <i>et al.</i> , 1995)
Non-mammalian systems, heterozygous length mutation	Saccharomyces cerevisiae (D32-3A heterozygous for 42- and 38-repeat-unit alleles of the human minisatellite MS32)	0, 1,000, 3,000, or 6,000 µg/mL for 42 hr (without metabolic activation)		+ (6,000 µg/mL)		(Appelgren <i>et al.</i> , 1999)
Non-mammalian systems, inter-chromosomal recombination	Saccharomyces cerevisiae (RS122)	Treated for 17 hr			+ (15,000 µg/mL)	(Schiestl <i>et al.</i> , 1997)
Unscheduled DNA synthesis	Rat (Primary hepatocytes)	0.02 to 20 µg/mL for 18 hr [without metabolic activation]		+ (max effective dose= 20 µg/mL)		(Althaus <i>et al.</i> , 1982)
Unscheduled DNA synthesis	Rat (Primary hepatocytes)	Incubation for 5 to 20 hours		- (50 µM or 17 µg/mL)		(Probst <i>et al.</i> , 1981)

Assay	Species (strain or cell type)	Study design	Aroclor 1242*	Aroclor 1254*	Aroclor 1260*	Reference
* Each row of the table represents a separate mutagenicity test. Population of the Aroclor column indicates the assay was performed using that Aroclor; - indicates a negative outcome [no mutagenicity]; + indicates a positive outcome; the value in parenthesis is the concentration for which the effect was observed (highest concentration in negative assays / lowest concentration for a positive test).						

Table A3-2. *In vivo* mutagenesis studies with Aroclors 1242, 1254, and 1260

Assay	Species, strain, sex (n/group)	Study design (cells)	Aroclor 1242*	Aroclor 1254*	Aroclor 1260*	Reference
Chromosomal aberrations	Mouse, Swiss albino, M (n=5)	1, 2, or 4 mg/kg/day i.p. for 5 weeks (Sperm)		+ (4 mg/kg/day)		(Attia <i>et al.</i> , 2014)
Chromosomal aberrations	Rat, SD, M (n=18)	Oral dose of 50 mg/kg/day for 7 days (Spermatogonial)		- (50 mg/kg po x7)		(Dikshith <i>et al.</i> , 1975)
Chromosomal aberrations	Rat, Holtzman, M (n=6-9)	Rats fed 0, 5, 50, or 500 ppm for 5 weeks (Bone marrow)		- (500 ppm in diet for 5 wks)		(Garthoff <i>et al.</i> , 1977)
Chromosomal aberrations	Rat, Holtzman, M (n=6-9)	Rats fed 0, 5, 50, or 500 ppm for 5 weeks (Spermatogonial)		- (500 ppm in diet for 5 wks)		(Garthoff <i>et al.</i> , 1977)
Chromosomal aberrations	Rat, Osborne- Mendel, M (n=8)	Single oral doses of 1,250, 2,500, or 5,000 mg/kg (Bone marrow)	- (5,000 mg/kg po x1)			(Green <i>et al.</i> , 1975a)
Chromosomal aberrations	Rat, Osborne- Mendel, M (n=8)	Single oral doses of 1,250, 2,500, or 5,000 mg/kg (Spermatogonial)	- (5,000 mg/kg po x1)			(Green <i>et al.</i> , 1975a)
Chromosomal aberrations	Rat, Osborne- Mendel, M (n=8)	Oral dose of 500 mg/kg/day for 4 days (Bone marrow)	- (500 mg/kg po x4)			(Green <i>et al.</i> , 1975a)
Chromosomal aberrations	Rat, Osborne- Mendel, M (n=8)	Oral dose of 500 mg/kg/day for 4 days (Spermatogonial)	- (500 mg/kg po x4)			(Green <i>et al.</i> , 1975a)
Chromosomal aberrations	Rat, Osborne- Mendel, M (n=8)	Oral dose of 75, 150, or 300 mg/kg/day for 5 days (Bone marrow)		- (300 mg/kg po x5)		(Green <i>et al.</i> , 1975a)
DNA adducts, 32P- postlabelling	Rat, F344, M (n=6)	25 mg/kg injected p.o. daily for 5 wks (Liver cells)		- (25 mg/kg/day x 35 days)		(Chadwick <i>et al.</i> , 1993)
DNA adducts, 32P- postlabelling	Rat, SD, M (n=3)	Two i.p. injections of 500 mg/kg, each given 2 weeks apart; sacrificed 2 and 6 weeks after second injection (Kidney cells)		- (500 mg/kg/day x 2 days, 2- wks apart)		(Nath <i>et al.</i> , 1991)
DNA adducts, 32P- postlabelling	Rat, SD, M (n=3)	Two i.p. injections of 500 mg/kg, each given 2 weeks apart; sacrificed 2 weeks after second injection (Liver cells)		- (500 mg/kg/day x 2 days, 2- wks apart)		(Nath <i>et al.</i> , 1991)

Assay	Species, strain, sex (n/group)	Study design (cells)	Aroclor 1242*	Aroclor 1254*	Aroclor 1260*	Reference
DNA adducts, 32P-postlabelling	Rat, SD, M (n=3)	Two i.p. injections of 500 mg/kg, each given 2 weeks apart; sacrificed 6 weeks after second injection (Lung cells)		+ (500 mg/kg/day x 2 days, 2-wks apart)		(Nath <i>et al.</i> , 1991)
DNA adducts, 32P-postlabelling	Rat, Lewis, M (n=6)	Administered single treatment of Aroclor 1242 by gavage; sacrificed 24 hr after oral dosing (Glandular stomach)	- (20 mg/kg po x1)			(Schilderman <i>et al.</i> , 2000)
DNA adducts, 32P-postlabelling	Rat, Lewis, M (n=6)	Administered single treatment of Aroclor 1242 by gavage; sacrificed 24 hr after oral dosing (Liver)	- (20 mg/kg po x1)			(Schilderman <i>et al.</i> , 2000)
DNA adducts, 32P-postlabelling	Rat, Lewis, M (n=6)	Administered single treatment of Aroclor 1242 by gavage; sacrificed 24 hr after oral dosing (Prostate)	- (20 mg/kg po x1)			(Schilderman <i>et al.</i> , 2000)
DNA adducts, 32P-postlabelling	Rat, Lewis, M (n=6)	Administered single treatment of Aroclor 1242 by gavage; sacrificed 24 hr after oral dosing (Seminal vesicles)	- (20 mg/kg po x1)			(Schilderman <i>et al.</i> , 2000)
DNA adducts, 32P-postlabelling	Rat, Lewis, M (n=6)	Administered single treatment of Aroclor 1242 by gavage; sacrificed 24 hr after oral dosing (Spleen)	- (20 mg/kg po x1)			(Schilderman <i>et al.</i> , 2000)
DNA adducts, 32P-postlabelling	Rat, Lewis, M (n=6)	Administered single treatment of Aroclor 1242 by gavage; sacrificed 24 hr after oral dosing (Testes)	- (20 mg/kg po x1)			(Schilderman <i>et al.</i> , 2000)
DNA adducts, 32P-postlabelling	Rat, Lewis, M (n=6)	Administered single treatment of Aroclor 1242 by gavage; sacrificed 24 hr after oral dosing (Thymus)	- (20 mg/kg po x1)			(Schilderman <i>et al.</i> , 2000)
DNA adducts, 32P-postlabelling	Mouse, B6C3F1/Crl BR, B (n=6)	Administered diet containing 200 ppm Aroclor 1260 for 2 weeks; sacrificed 24 hr after last diet administration (Liver)			- (200 ppm in feed for 2 wks)	(Whysner <i>et al.</i> , 1998)
DNA adducts, 32P-postlabelling	Mouse, B6C3F1/Crl BR, B (n=6)	Administered single oral dose of 50 mg/kg; sacrificed 24 hr after gavage (Liver)			- (50 mg/kg po x1)	(Whysner <i>et al.</i> , 1998)
DNA adducts, 8-OHdG	Rat, Wistar, Both (n=6)	pregnant rats gavaged with 1 mg/kg bw from second week of gestation to PND21. (Cerebellum)		+ (1 mg/kg bw)		(Dogan and Alcigir, 2019)
DNA adducts, 8-OHdG	Rat, Wistar, Both (n=6)	pregnant rats gavaged with 1 mg/kg bw from second week of gestation to PND21. (Cerebral cortex)		+ (1 mg/kg bw)		(Dogan and Alcigir, 2019)

Assay	Species, strain, sex (n/group)	Study design (cells)	Aroclor 1242*	Aroclor 1254*	Aroclor 1260*	Reference
DNA adducts, 8-OHdG	Rat, Wistar, Both (n=6)	pregnant rats gavaged with 1 mg/kg bw from second week of gestation to PND21. (Plasma)		+ (1 mg/kg bw)		(Dogan and Alcigir, 2019)
DNA adducts, 8-OHdG	Rat, Lewis, M (n=6)	Administered single treatment of Aroclor 1242 by gavage; sacrificed 24 hr after oral dosing (Glandular stomach)	- (20 mg/kg po x1)			(Schilderman <i>et al.</i> , 2000)
DNA adducts, 8-OHdG	Rat, Lewis, M (n=6)	Administered single treatment of Aroclor 1242 by gavage; sacrificed 24 hr after oral dosing (Liver)	- (20 mg/kg po x1)			(Schilderman <i>et al.</i> , 2000)
DNA adducts, 8-OHdG	Rat, Lewis, M (n=6)	Administered single treatment of Aroclor 1242 by gavage; sacrificed 24 hr after oral dosing (Prostate)	- (20 mg/kg po x1)			(Schilderman <i>et al.</i> , 2000)
DNA adducts, 8-OHdG	Rat, Lewis, M (n=6)	Administered single treatment of Aroclor 1242 by gavage; sacrificed 24 hr after oral dosing (Seminal vesicles)	- (20 mg/kg po x1)			(Schilderman <i>et al.</i> , 2000)
DNA adducts, 8-OHdG	Rat, Lewis, M (n=6)	Administered single treatment of Aroclor 1242 by gavage; sacrificed 24 hr after oral dosing (Spleen)	- (20 mg/kg po x1)			(Schilderman <i>et al.</i> , 2000)
DNA adducts, 8-OHdG	Rat, Lewis, M (n=6)	Administered single treatment of Aroclor 1242 by gavage; sacrificed 24 hr after oral dosing (Testes)	- (20 mg/kg po x1)			(Schilderman <i>et al.</i> , 2000)
DNA adducts, 8-OHdG	Rat, Lewis, M (n=6)	Administered single treatment of Aroclor 1242 by gavage; sacrificed 24 hr after oral dosing (Thymus)	- (20 mg/kg po x1)			(Schilderman <i>et al.</i> , 2000)
DNA deletion (intrachromosomal recombination / frequency of DEL recombination)	Mouse, C57BL/6J p(un)/p(un), (n=8-32)	Exposed in utero at GD 10.5 (i.p. injection to dams) (Evaluated fur color (i.e., spots) on offspring)			+ (500 mg/kg ip x1)	(Schiestl <i>et al.</i> , 1997)
DNA strand breaks (comet assay)	Mouse, Swiss albino, M (n=5)	1, 2, or 4 mg/kg/day i.p. for 5 weeks (Sperm)		+ (4 mg/kg/day)		(Attias <i>et al.</i> , 2014)
Dominant lethal mutation	Mouse, Charles River albino, M (n=12)	Single injection (i.p.) 0, 500, or 1,000 mg/kg and mated immediately after dose administration and weekly thereafter for 6 weeks	- (1,000 mg/kg, 1x)			(Arnold, 1972a)
Dominant lethal mutation	Mouse, Charles River albino, M (n=12)	Single injection (i.p.) 0, 500, or 1,000 mg/kg and mated immediately after dose administration and weekly thereafter for 6 weeks		- (1,000 mg/kg, 1x)		(Arnold, 1972b)

Assay	Species, strain, sex (n/group)	Study design (cells)	Aroclor 1242*	Aroclor 1254*	Aroclor 1260*	Reference
Dominant lethal mutation	Mouse, Charles River albino, M (n=12)	Single injection (i.p.) 0, 500, or 1,000 mg/kg and mated immediately after dose administration and weekly thereafter for 6 weeks			- (1,000 mg/kg, 1x)	(Arnold, 1972c)
Dominant lethal mutation	Rat, Osborne-Mendel, (n=10)	Daily oral intubation doses of 125 or 250 mg/kg/day for 5 days; males were mated one additional week (a total of 11 wks); females killed mid-pregnancy	- (250 mg/kg/day po x 5)			(Green <i>et al.</i> , 1975b)
Dominant lethal mutation	Rat, Osborne-Mendel, (n=10)	Single oral intubation doses of 625, 1250, or 2500 mg/kg; each male was mated with 2 females weekly for the following 10 wks; females killed mid-pregnancy	- (2500 mg/kg po x 1)			(Green <i>et al.</i> , 1975b)
Dominant lethal mutation	Rat, Osborne-Mendel, (n=10)	Daily doses of 75 or 150 mg/kg/day for 5 days; males were mated one additional week (a total of 11 wks); females killed mid-pregnancy		- (150 mg/kg/day po x5)		(Green <i>et al.</i> , 1975b)
Dominant lethal mutation	Rat, Osborne-Mendel, (n=10)	Daily doses of 75, 150, or 300 mg/kg/day for 5 days; each male was mated with 2 females weekly for the following 10 wks; females killed mid-pregnancy		- (300 mg/kg/day po x5)		(Green <i>et al.</i> , 1975b)
Gene mutation	Mouse, BigBlue (lambda/lacI transgenic C57BL/6), M (n=6)	100 ppm in diet for 7 wks (Liver cells)		(+) 100 ppm in diet for 7 wks		(Davies <i>et al.</i> , 2000)
Gene mutation (minisatellite PC-2)	Mouse, C57Bl/6, M (n=2 litters from each mating time-point)	Treatment of 100 mg/kg via i.p. injection 2x (12 days apart). Mating occurred 7 and 25 days following last injection. Offspring sacrificed at 3 weeks of age. (Liver)		- (100 mg/kg i.p. 2x)		(Hedenskog <i>et al.</i> , 1997)
Gene mutation (minisatellite PC-1)	Mouse, C57Bl/6, M (n=2 litters from each mating time-point)	Treatment of 100 mg/kg via i.p. injection 2x (12 days apart). Mating occurred 7 and 25 days following last injection. Offspring sacrificed at 3 weeks of age. (Liver)		+ (100 mg/kg i.p. 2x)		(Hedenskog <i>et al.</i> , 1997)

Assay	Species, strain, sex (n/group)	Study design (cells)	Aroclor 1242*	Aroclor 1254*	Aroclor 1260*	Reference
Micronucleus formation	Mouse, C57BL/6 x C3H/He F1, F (n=8)	Mice injected i.p. 1x/day for 5 consecutive days; sacrificed ~4 hrs after last injection (Bone marrow)		- (15,000 mg/kg x 5)		(Bruce and Heddle, 1979)
Non-mammalian systems, Chromosomal aberrations	Fish, C. idella, - (n=4)	0, 50, 150, 300 mg/kg in corn oil (i.p.); evaluated after 48 hr (Kidney cells)		+ (50 mg/kg)		(Al-Sabti, 1985)
Non-mammalian systems, Chromosomal aberrations	Fish, Cyprinus carpio (carp), - (n=5-6)	0, 50, 150, 300 mg/kg in corn oil (i.p.); evaluated after 48 hr (Kidney cells)		+ (50 mg/kg)		(Al-Sabti, 1985)
Non-mammalian systems, Chromosomal aberrations	Fish, T. tinca, - (n=4)	0, 50, 150, 300 mg/kg in corn oil (i.p.); evaluated after 48 hr (Kidney cells)		+ (50 mg/kg)		(Al-Sabti, 1985)
Non-mammalian systems, Chromosomal aberrations	Chicken, Gallus domesticus, - (n=30 cnrls 64 with 10 ppm, & 57 with 20 ppm)	0, 0.5 or 1.0 mg injected into White Leghorn eggs and incubated for 4-5 days	- (1.0 mg/egg)			(Blazak and Marcum, 1975)
Non-mammalian systems, Micronucleus formation	Fish, Cyprinus carpio (carp), - (n=5-6)	0, 50, 150, 300 mg/kg in corn oil (i.p.); evaluated after 48 hr (Erythrocytes)		+ (50 mg/kg)		(Al-Sabti, 1986)
Sperm morphology	Mouse, C57BL/6 x C3H/He F1, M (n=8)	Mice injected i.p. 1x/day for 5 consecutive days; sacrificed 35 days after last injection (Sperm)		- (15,000 mg/kg x 5)		(Bruce and Heddle, 1979)
Unscheduled DNA synthesis	Monkey, Cynomolgus, M (n=2)	50 mg/kg i.p. ~24 hr prior to sacrifice (Primary hepatocytes)		- (50 mg/kg ip x1)		(Hamilton <i>et al.</i> , 1997)
Unscheduled DNA synthesis	Monkey, Cynomolgus, M (n=1)	50 mg/kg i.p. 2x; first injection ~24 hr prior to sacrifice and 2nd injection ~17 hr prior to sacrifice (Primary hepatocytes)		- (50 mg/kg ip x2)		(Hamilton <i>et al.</i> , 1997)
Unscheduled DNA synthesis	Rat, SD, M (n=3)	300 mg/kg i.p. 5 days prior to sacrifice (Primary hepatocytes)		- (300 mg/kg ip x1)		(Kornbrust and Dietz, 1985)
Unscheduled DNA synthesis	Rat, SD, M (n=ns)	500 mg/kg i.p. 1 day prior to sacrifice (Primary hepatocytes)		- (500 mg/kg ip x1)		(Shaddock <i>et al.</i> , 1989)

* Each row of the table represents a separate mutagenicity test. Population of the Aroclor column indicates the assay was performed using that Aroclor; - indicates a negative outcome [no mutagenicity]; + indicates a positive outcome; the value in parenthesis is the concentration for which the effect was observed (highest concentration in negative assays / lowest concentration for a positive test).

B. Analysis of two-year bioassays conducted on PCBs and specific PCB congeners.

1. Evaluation of PCBs for carcinogenic activity in laboratory animal bioassays:

Both the International Agency for Research on Cancer (IARC) and the US EPA have designated mixtures of PCBs to be potentially carcinogenic to humans, based on the appearance of liver tumors following exposure of laboratory animals to Aroclor mixtures for extended periods of time (usually 2 years). Indeed, since the first Monsanto-sponsored 2-year chronic bioassay of Aroclors in the late 1960s, there have been over a dozen different laboratory 'life time' or near life-time bioassays in rodents. Most of these have used rats, but several studies in mice are also available. A summary of each of these studies is provided below. A substantive analysis of the collective data then follows.

IBT (1971b)- Monsanto contract study on Aroclor 1254

50 male and 50 female albino (Charles River; presumably Sprague-Dawley strain) rats per group, at 0, 1, 10, and 100 ppm in the diet. Five animals from each group were sacrificed at 3, 6, 12 months, leaving approximately 35 animals in the 2 yr completion. A table in this paper shows the mortality curves for Aroclor 1254 and control animals. Even though the group size for the 2 year study was relatively large (35 males and 35 females after scheduled interim sacrifices at 3, 6 and 12 months), only 26 controls (10 males, 16 females; 37% survival) and 20 high dose (8 males, 12 females) were alive at the end of the 2 year study. These survival values are similar to the 2 year survival of Sprague-Dawley used as controls in NTP studies (Dinse *et al.*, 2010); two year survival for S-D rats averaged 42% (range, 28-50%, across 9 studies); 2 year survival in Fisher 344 rats was substantially greater, with an average of 67% alive at 2 years (range 60-76%).

There was a modest, but statistically significant, increase in liver weight at 24 months in the 100 ppm males, but not females. This was not seen in the two lower doses at the three or six-month sacrifice. At the end of the two-year study, liver weight was significantly increased in both males and females in the high dose 100 ppm group, but no change at the 1 or 10 ppm group. Histopathology of the liver in all groups, except for the high dose males and females at 24 months, were unremarkable. In the high dose males at 24 months, of the 11 animals still alive and subjected to necropsy, the following pathology was observed (Table A3-3).

It should be noted that, although Monsanto had contracted in good faith with IBT to conduct what at the time was considered to be 'state-of-the-art' 2-year chronic bioassays for carcinogenesis, IBT was subsequently found to have falsified data in other studies of pharmaceuticals, thus bringing in to question the validity of these studies, and I do not rely on these studies to support or refute the carcinogenic activity of certain Aroclor mixtures in laboratory rats. However, the reported results of the IBT studies were consistent with the subsequent finding of the properly conducted NCI 1978 2-year bioassay of Aroclor 1254, which was that "*Aroclor 1254 was not carcinogenic to the rats under the test conditions*" (National Institutes of Health, 1978).

Table A3-3. Histopathology of the liver of rats treated with Aroclor 1254

	Control		1 ppm		10 ppm		100 ppm	
	Male	Females	Male	Females	Male	Females	Males	Females
	N=11	N=14	N=11	N=22	N=10	N=12	N=11	N=14
Focal degeneration	1	1	0	0	1	0	2	0
Vacuolization (Fatty)	0	2	1	3	3	1	3	10
Necrosis	0	0	1	1	0	0	0	0
Hyperplasia (ductal)	2	2	1	2	1	2	3	1
Hyperplasia	0	0	0	0	0	0	0	1
hypertrophy	0	0	0	0	1	0	3	6
Nodular hyperplasia	0	0	0	0	0	1	0	1
Focal lymph infiltrate	0	3	0	3	0	4	1	0
Fibrosis	0	0	0	0	0	0	0	0
Hepatoma (adenoma)	0	0	0	0	0	1	0	1
Carcinoma/sarcoma	0	0	0	0	0	0	0	0

There were no data suggesting PCBs were carcinogenic in the liver (or anywhere else) in this study. No carcinomas were seen, and only two benign tumors in the liver were identified, one in the 10 ppm and one in the 100 ppm dose (both females). The authors concluded that Aroclor 1254 induces fatty infiltration in some of the animals, but that there was no evidence of any kind of carcinogenic effect of Aroclor in this study.

Aroclor 1242 (IBT, 1971a)—exactly the same design, with much the same findings as for 1254- some fatty infiltration at the high dose, but no hepatomas or nodular hyperplasia was found in any animal in this study.

Aroclor 1260 (IBT, 1971c)—exactly the same design, with much the same findings as for 1254- some fatty infiltration at the high dose, but no hepatomas or nodular hyperplasia was found in any animal in this study, as with 1242.

Ito et al. (1973b)

Male mice were used, 12 per dose and treatment group. 3 doses (100, 250, 500 ppm) of each of 3 Kanechlors (100, 300, 500). Animals were treated for 32 weeks. Five out of twelve animals in the high dose Kanechlor 500 group had hepatocellular carcinoma, and 7 had nodular hyperplasia. No animals in any of the other dose /treatment groups had tumors or nodules. They also looked at interactions between benzene hexachloride and Kanechlor 500 and found that 250 ppm Kanechlor 500 increased tumor yield in animals also give 250 ppm BHC.

Ito et al. (1973a)

Essentially the same study as above, reported at a scientific symposium. Some additional groups of BHC-treated animals are present in this paper, but the PCB-only data appears to be the same as in the JNCI paper.

Ito et al. (1974)

Male Wistar strain of rats were used, with 10 -25 animals per dose group. Kanechlor 300, 400 and 500 were used at 3 doses, 100, 500 and 1000 ppm. Animals were fed PCBs in the diet for approximately 1 year (Most groups were treated for 52 weeks, but some were less; for example, Kanechlor 500 group was only treated for 27 weeks). No liver tumors were found, but there was evidence of preneoplastic change and hepatotoxicity in some animals. Nodular hyperplasia was present in a dose-related manner in the Kanechlor 500 group (12, 31, 39%), but seen sporadically in the other two, although the sample sizes were smaller.

Kimbrough et al. (1975)

Sherman strain of Female rats (N=200) were fed 100 ppm of Aroclor 1260 for approximately 21 months, then sacrificed at 23 months. Controls (N=200) received the same diet with corn oil. At the end of the study, 184 PCB-treated rats were still alive, and 26 had hepatocellular carcinomas (14%). One of the 173 control rats alive at 23 months had hepatocellular carcinoma. 148 of the 185 PCB-treated rats had preneoplastic nodules, and nearly all of the females in the PCB-treated group had preneoplastic foci in their livers at sacrifice, compared to areas of hepatocellular alteration in 28 of the 173 controls. The incidence of mammary adenocarcinoma was reduced in the PCB treated rats (5 in controls, 1 in the treated), as well as reductions in thyroid and pituitary tumors, and this may explain at least some of the increased survival in the Aroclor-treated females.

There was no indication as to when the earliest liver tumors occurred, as they did not apparently necropsy the animals that died prior to the end of the 23 month study.

There were two lymphomas noted in the treated group, and none in the controls. It was concluded that Aroclor 1260 is hepatocarcinogenic in female Sherman rats.

NCI Bioassay- Moore et al. (1994); Morgan et al. (1981); NCI (1978); Ward (1985)

24 Fisher 344 rats in each group (males, females, 3 doses plus control – doses 25, 50, 100 ppm in diet) for 2 years (104-105 weeks). The incidence of liver adenomas + carcinomas was elevated, but not statistically significant, in the highest dose group (1/24 in Control, 3/24 100 ppm males, 1/24 Control, 2/24 100 ppm, females). There was also a high incidence of hyperplastic nodules in the rats, which the authors concluded was treatment related. There were also 2 adenocarcinomas of the stomach in males and females in the high dose groups (2 and 2), not seen in controls, and a low incidence in historical controls. Authors of the NCI study stated in their conclusions: "It is concluded that, under the conditions of this study, Aroclor 1254 was not carcinogenic in Fisher 344 rats; however, a high incidence of hepatocellular proliferative lesions was observed in both male and female rats was related to the administration of the chemical. In addition, the carcinomas of the intestinal tract may be associated with administration of Aroclor 1254 to both male and female rats." In discussions of the peer review comments, these conclusions were approved, and a motion was made and adopted that added the following statement: "Based on the liver proliferative lesions in the treated animals and published reports, it is suggested that Aroclor 1254 may be a tumor promoter." Six of the eight reviewers voted in favor of the motion, 1 objected, and 1 abstained.

Results from this study were also published in two other papers, one by Morgan *et al.* (1981), and one by Ward (1985). In the Morgan *et al.* (1981) report of this study, they collected the stomachs and analyzed for pathology. In males, they found 2/24, 2/24, 4/24 and 7/24 rats with 'stomach lesions' of some sort, at 0, 25,

50 and 100 ppm, respectively. For females, the incidence of stomach lesions was 1/23, 3/24, 4/24 and 10/24. Histological analyses of the stomach lesions were described as "focal metaplasia" with lymphocyte infiltration and other inflammatory cells noted, suggesting that there was significant inflammation and necrosis. A total of 6 adenocarcinomas were found out of the 291 rats that were analyzed at the end of the study. The adenocarcinomas were: 0/47, 1/48, 3/48 and 2/48 at 0, 25, 50 and 100 ppm Aroclor 1254. Ward also reported liver histopathology results reported in the NCI report. As seen in Table A3-5, 2 rats in the high dose (100 ppm) male group had hepatocellular carcinoma, and 5 others had adenomas. Two adenomas were found in the mid-dose (50 ppm) and 1 in the low dose (25 ppm) group. No hepatocellular carcinomas were seen in these Fisher 344 female rats. Three adenomas were seen in the mid-dose and 2 were reported in the high dose group. No adenomas were seen in the controls or the low dose group of females. Ward states that he looked for correlations between liver tumors and the gastric metaplasia and adenocarcinomas, but found no correlation between animals with gastric metaplasia and those with liver tumors.

Table A3-4. Hepatocellular foci and tumors in groups 24 F344 rats fed diets containing Aroclor 1254 (from: Ward, 1985)

Dose, ppm	Sex	Hepatocellular foci/cm ² ± SD			Hepatocellular adenoma (number with lesion)			Carcinoma	Any liver tumor
		Eosinophilic	Basophilic (% with lesion)	Vacuolated	Eosinophilic	Basophilic	Vacuolated		
0	Male	0 (0)	1.48 ± 3.75 (29)	0 (0)	0	0	0	0	0
25	Male	0.93 ± 1.82* (29)†	0.27 ± 1.03 (8)	0.60 ± 1.80 (16)	1	0	0	0	1
50	Male	0.76 ± 1.29 (33)†	0.63 ± 1.70 (16)	0.58 ± 1.70 (16)	2	0	0	0	2
100	Male	1.24 ± 2.84* (43)†	1.58 ± 3.24 (37)	0.41 ± 0.66 (29)†	3	1	1	2	7*
0	Female	0 (0)	1.15 ± 3.30 (29)	0.09 ± 0.45 (4)	0	0	0	0	0
25	Female	2.74 ± 3.86* (54)†	0.44 ± 0.92 (20)	0.04 ± 0.21 (4)	0	0	0	0	0
50	Female	3.34 ± 3.49† (62)†	0.15 ± 0.73 (4)	0.10 ± 0.50 (4)	3	0	0	0	3
100	Female	1.27 ± 2.30* (41)†	0.87 ± 2.31 (29)	1.07 ± 1.62* (41)†	1	0	1	0	2

* < 0.05 as compared with appropriate control.

† < 0.01 as compared with appropriate control.

Schaeffer et al. (1984)

This study used only male Wistar rats, and also used a formulation of PCB manufactured by a German company (i.e., not Monsanto PCBs). Male rats were administered 100 ppm Clophen A30 and A60 fed for up to 800 days. There were no HCCs in 132 control animals at 800 days (27 months), but there was a significant increase in animals dying from hepatocellular carcinomas in the A60 group (9/129, or 7%), but not A30 (1/138) at 800 days (27 months). However, deaths from non-liver tumors were reduced, most notably thymomas, which were present in 17% of controls, but none in A60 group. At the end of the study, there was a total of 93 tumors (73%) seen in 131 control animals available for necropsy. In the Clophen A30 group there were 75 tumors (55%) seen in 138 necropsied animals (only 1 HCC), and in the A60 group there were 94 (73%) out of 129 necropsied animals that had any kind of tumor, but most (63) were HCC. Thus, nearly half of the animals in the A60 group had liver tumors, but they generally did not die early from this (9 out of 85 animals that died before the end of the experiment had liver tumors, and all of those were in the last 100 days prior to the end of the 800 day study, or ~23 months). There were far fewer non-HCC tumors in the

Clophen A60 group (24.5% vs. 60% in controls had non-liver tumors). Thus, overall, PCB treatment had no significant effect on overall tumorigenesis, even though HCC was dramatically elevated in the A60 group. The incidence of urogenital track pathologies, including prostatitis and nephritis, were greatly reduced in the treated groups, relative to the controls.

Norback and Weltman (1985)

Sprague-Dawley rats, 70 per group (males and females), were fed Aroclor 1260 at 100 ppm for 16 months and then switched to 500 ppm for 8 months, then to a control diet for another 5 months of the 29 month study. The control group consisted of 63 males and 63 females. Three animals from each group (2 from controls) were sacrificed at 1, 3, 6, 9, 12, 15 and 18 months and the livers were evaluated for histopathology. The remaining 49 animals completed the protocol (or died prematurely; 46 males and 47 females were alive at the end of the study). At the end of the study, no tumors were reported in either the control males or females. In the Aroclor 1260 treated males, five males had neoplastic nodules, and 2 had trabecular carcinoma, with 39 of the 46 males exhibiting no liver pathology. In contrast, females showed substantially greater susceptibility to Aroclor 1260. 43 of 47 female rats (91%) either trabecular carcinoma (19, or 40%) or adenocarcinoma or adenocarcinoma+trabecular cell carcinoma (24, or 51%). Two females had neoplastic nodules but no carcinomas, and 2 had no evidence of any liver neoplasia. Of the 24 male and 24 females sacrificed in the interim periods, none of the males had any liver cancers, and only 1 had nodules (at 24 mo). Of the females, 5 had trabecular cell carcinomas (1 at 15, 2 at 18 and 2 at 24 mo), and 2 had adenocarcinomas (both at 24 mo). Ten of the females in the interim sacrifice group had nodules, with the earliest appearing at 12 months. The authors concluded that Aroclor 1260 induced a significant level of both trabecular cell carcinoma and adenocarcinoma of the liver in female rats. Males were substantially less sensitive. The authors noted that, although the tumors shared morphological characteristics of malignant tumors, "their biological behavior was relatively unaggressive." They noted that there were no metastatic tumors, and that mortality was not increased. They concluded that the lack of greater morbidity and mortality was due to "slow progression of the neoplastic nodules and their late appearance and slow growth of the hepatocellular carcinoma."

Moore et al. (1994)

Because the US EPA had begun using these studies to establish regulatory guidelines for all PCBs, the National Toxicology Program organized a "Pathology Working Group" (PWG) consisting of 5 independent, Board certified Veterinary Pathologists to review the original histopathology (microscopic evaluation of tissues from each of the animals) from 4 data sets (Kimbrough *et al.*, 1975; NCI, 1978; Norback and Weltman, 1985; Schaeffer *et al.*, 1984) that included 7 different bioassays for various Aroclors. The consensus report was published by Moore *et al.* (1994).

They reported that the reevaluation of the histopathology of all seven studies found relatively little difference in pathological diagnoses from the original study, with a few exceptions, as shown in Tables 4 and 5 from their report (Table A3-5 and Table A3-6).

Table A3-5. Comparison of Liver Neoplasms in Male Rats fed a PCB Mixture Containing 60% Chlorine (from: Moore *et al.*, 1994)

Strain Group Pathology diagnoses	Wistar ^a				Sprague-Dawley ^b			
	Control		Treated		Control		Treated	
	Orig	Reeval	Orig	Reeval	Orig	Reeval	Orig	Reeval
No. examined	131	120	129	125	32	31	46	40
Benign hepatic tumors	5 ^c	6 ^d	62 ^c	47 ^d	0	0 ^d	5 ^c	4 ^d
Hepatocellular carcinomas	1	2	61	67	0	0	2 ^e	1
Benign + carcinomas ^f	6	8 ^g	123	114	0	0	7	5

^a Schaeffer *et al.* (1984).

^b Norback and Weltman (1985).

^c Reported as neoplastic nodules.

^d Reported as hepatocellular adenomas.

^e Original study reported as trabecular carcinomas.

^f Sum of benign hepatocellular tumor or hepatocellular carcinoma. If rat had both, then lesions were counted only as carcinoma.

^g One cholangiocarcinoma also diagnosed in a rat with other liver tumors.

Table A3-6. Comparison of Liver Neoplasms in Male Rats fed a PCB Mixture Containing 42% Chlorine (from: Moore *et al.*, 1994)

Strain Group Pathology diagnoses	Wistar ^a			
	Control		Treated	
	Orig	Reeval	Orig	Reeval
No. examined	131	120	130	128
Benign hepatic tumors	5 ^b	6 ^c	38 ^b	14 ^c
Hepatocellular carcinomas	1	2	4	2
Benign + carcinomas ^d	6	8 ^e	42	16

^a Schaeffer *et al.* (1984).

^b Reported as neoplastic nodules.

^c Reported as hepatocellular adenomas.

^d Sum of benign hepatocellular tumor or hepatocellular carcinoma. If rat had both, then lesions were counted only as carcinoma.

^e One cholangiocarcinoma also diagnosed in a rat with other liver tumors.

There were relatively modest differences in pathological diagnoses between the PWG's analyses and that of the original authors for Aroclor 1260. However, there was a substantial difference between the PWG evaluation and that of the Schaeffer *et al.* (1984) report on Aroclor 1242. Whereas Shaeffer *et al.* reported 42 hepatocellular tumors (benign + malignant) in the Aroclor 1242-treated group, with only 6 tumors in the controls, the PWG found only 16 benign+ malignant liver tumors in the treated group, and 8 in the controls, making the original analysis of this study somewhat questionable in terms of the tumorigenicity of Aroclor 1242.

The final conclusion of the PWG's reevaluation of these studies was:

"The reevaluations permit a more confident comparison of the carcinogenic responses observed in seven different studies with PCBs. The results highlight three issues: PCBs with 60% chlorine content consistently provoke a high yield of liver tumors in rats; the liver tumor response observed in rats exposed to PCBs with lower levels of chlorine was not observed to have an increase in liver tumors; no clear sensitivity differences in tumor response were observed between males and females. These data indicate that continuation of a science policy of assuming that all PCBs are probable human carcinogens and possess a carcinogenic potency equivalent to the mixture that contains 60% chlorine has no scientific foundation and should be reconsidered."

Rao and Banerji (1988); Rao and Banerji (1990)

This study was a short-term exposure of two groups of 32 male Wistar rats exposed to 50 or 100 ppm Aroclor 1260 for 120 days in the diet. Animals were examined after 120 days. They report a high incidence (75% and 50% in the 50 and 100 ppm, respectively, groups) of what they refer to as 'Neoplastic Nodules', and based on these responses, titled the paper 'Induction of Liver Tumors in Male Wistar Rats.....' However, the description and the time frame was much more consistent with hyperplasia, as described by (Mayes *et al.*, 1998) and are non-neoplastic lesions, although these are often considered to potentially be 'preneoplastic' lesions. The quality of this study is questionable, and it provide little useful information. These authors also reported in a different paper (Rao and Banerji, 1990) from the same study histopathological changes in the liver, and again refer to these changes as 'neoplasia.'

Mayes et al. (1998) (this is the same study as the Brunner et al. (1997) study cited in the EPA 1996 Risk Assessment of PCBs)

This is, by far, the most substantial carcinogenicity bioassay for PCB mixtures ever done. This study involved 4 different commercial PCBs: Aroclor 1016 (0, 50, 100, 200 ppm), Aroclor 1242 (0, 50, 100), Aroclor 1254 (0, 25, 50, 100 ppm) and Aroclor 1260 (0, 25, 50, 100 ppm). PCBs were administered in the diet over 24 months, with careful records of dietary consumption, weight gain, etc. The study used 50 animals per dose per sex in each group, with 100 animals each in male and female control groups. Overall, PCB treatment did not result in treatment-related reduced mortality. There was a slightly increased mortality in Aroclor 1016 100 ppm males and Aroclor 1254 50 ppm males, but there were no dose trends, so it was not possible to conclude that this was treatment related. Conversely, there was a decrease in mortality in females in all PCB groups, and

this was statistically significant for all Aroclor 1016 and 1242 groups, and for the 25 ppm Aroclor 1254 and 100 ppm Aroclor 1260 females.

In males, there was a modest but significant increase in thyroid gland tumors, but these were benign adenomas. There was no increase in thyroid gland adenocarcinomas in any of the males or females. The increase in thyroid adenomas in males is likely explained by induction of P450s that reduce circulating thyroid hormones (T4 and T3) and thus induce thyroid hyperplasia (due to an increase in TSH in a feedback-regulated response to reduced T3 and T4). This is normally more common in males than females because of differences in baseline circulating levels of T4 and T3 in males.

In males, there was a significant increase in hepatocellular adenomas in the high dose 1260 group, but no increase in either liver adenomas or carcinomas in any of the other PCB-treated males.

Female rats were more susceptible to the hepatocarcinogenic effects of PCBs than males (as seen in other studies using Sprague-Dawley strain of rats), with statistically significant increased levels of hepatocellular carcinomas in the mid (4/50) and high (6/50) dose 1254 and high (5/60) dose 1260-treated females. There were also a few cholangiocellular carcinomas in the mid dose 1254 (6/50) and high dose (3/50) 1260 female groups. There were a large number of hepatic adenomas in females in most of the PCB-treated females that increased in a dose-related fashion, except for Aroclor 1016 (in the 1016 females, there was 10% of the animals in the mid and high dose groups with adenomas, and one hepatocellular carcinoma in the 100 ppm dose group).

This study demonstrated that higher chlorinated PCBs (1254, 1260) are effective liver carcinogens in females, which supports previous studies showing that higher chlorinated PCBs are hepatocarcinogenic, and that females are more sensitive than males. However, there may also be significant strain differences in susceptibility since the NCI/Moore *et al.* and the Morgan *et al.* study had a substantially lower incidence of liver tumors in female Fisher 344 rats compared to Mayes studies that used Sprague-Dawley rats.

This study also demonstrated a significant reduction in female breast tumors in Aroclor 1242, 1254 and 1260-treated female rats. This is consistent with previous studies (Kimbrough *et al.*, 1975) in Sherman rats (which have a high rate of background mammary carcinomas) and may also explain the increased survival of PCB-treated female rats seen in this study.

NTP (2006c)- PCB126 Gavage study

PCB126 is the most potent agonist of the rat AhR, e.g., the most 'dioxin-like' of all PCBs. Although it does not contribute significantly to the total percentage of PCB congeners (less than 0.1%), because of its very high affinity for the rat Arylhydrocarbon Receptor (AhR) it contributes significantly to the total 'dioxin-like equivalents' (TEQs) in commercial mixtures of higher chlorinated PCBs. It's use in this experimental model was focused more on understanding the role of AhR agonists and the value of the 'TEF' approach for risk assessment of dioxins and dioxin-like chemicals than on risk assessment for PCBs. This is one of a series of NTP studies focused on evaluating the validity of the TEF approach for dioxins and other dioxin-like HPAHs. The study notes: "The design of these studies on PCB126 should be considered within the context of the dioxin TEF evaluation."

PCB126 was administered by gavage to female Harlan Sprague-Dawley rats (N=50 per group), 5 days/week for two years, at doses of 30, 100, 175, 300, 550, or 1,000 nanograms (ng) per kilogram of body weight. "A

group of 81 vehicle control female rats received the corn oil/acetone vehicle alone. A group of 28 rats received 10 ng/kg for up to 53 weeks only. Up to 10 rats per group were evaluated at 14, 31, or 53 weeks. A stop-exposure group of 50 female rats was administered 1,000 ng/kg PCB126 in corn oil:acetone (99:1) by gavage for 30 weeks then the vehicle for the remainder of the study.”

There were many effects on the liver, including significant increases in cholangiocellular carcinoma and cholangiocellular sarcomas, and a few hepatocellular adenomas, but no hepatocellular carcinomas were reported, in contrast to previous studies with mixtures of PCBs. There was an increase in a number of benign tumors in other tissues (oral mucosa, lung) with small but significant increase in squamous cell carcinoma of the lung and gingival squamous cell carcinoma.

As seen with some studies with Aroclors, there was a reduced incidence of mammary tumors in this study. Adenocarcinoma in the breast was reduced from 16% in the control to only 3% in the high dose group. Fibroadenomas were reduced in a similar way (from 86% in controls to 31% in high dose group). Pituitary tumors were also significantly decreased in the treated groups.

NTP (2006a)- PCB153

This study was of similar design to the PCB126 study above. However, the doses were more than 1000-fold higher. PCB153 is a non-DL PCB that is present in relatively high concentrations in humans, so that is the primary reason it was selected. It has no ‘dioxin-like’ activity (e.g., it has no AhR agonist activity, and thus is a ‘non-dioxin like’ PCB).

“Female Harlan Sprague-Dawley rats were administered PCB153 (greater than 99% pure) in corn oil:acetone (99:1) by gavage for 14, 31, or 53 weeks or 2 years. Groups of 80 (3,000 µg PCB153/kg body weight), 81 (100, 300, and 1,000 µg/kg), or 82 (10 µg/kg) female rats received PCB153 in corn oil:acetone (99:1) by gavage at doses of 10, 100, 300, 1,000, or 3,000 µg/kg 5 days per week for up to 105 weeks; a group of 81 female rats received the corn oil:acetone (99:1) vehicle alone. A stop-exposure group of 50 female rats was administered 3,000 µg/kg for 30 weeks and then the vehicle for the remainder of the study.”

In contrast to PCB126, PCB153 had very few effects on any organ system. There were 3 cholangiomas reported in the treated groups; only one hepatocellular adenoma was seen in the high dose (3,000 µg/kg/d) group, but no carcinomas in the liver or any other tissue were seen in excess in the treated groups. However, as with PCB126, there was a substantial reduction in the incidence of breast adenocarcinoma, from 15% in controls to 4% in the high dose group. However, there was no effect on fibroadenomas (42% in control, 47% in high dose).

NTP (2006d)- Combination of PCB126 and 153

This study looked at interaction of the two PCBs studied individually, as discussed above. Design was: Groups of 53 female rats were exposed by oral gavage to mixtures of PCB126 and PCB153 dissolved in corn oil five days a week for two years. Daily doses were 10, 100, 300, or 1,000 nanograms of PCB126, each with 1,000 times as much PCB153, per kilogram body weight. Animals receiving the corn oil alone served as the control group. There were two hepatocellular carcinomas in the high dose group, and numerous (26 out of 103 animals; 21 in the high dose and 5 in the next lower level) cholangiocellular carcinomas in the two highest dose groups (300 and 1,000 ng/kg-d PCB126 and 300 and 1,000 µg/kg PCB153). There were 16 (out of 53 animals) hepatocellular adenomas in the highest dose group (1,000 ng/kg of PCB126 plus 1,000 µg/kg

of PCB153). This study showed that PCB153 does not antagonize many of the toxic effects seen with PCB126, as had previously been hypothesized. The number of liver tumors seen in the female rats given the two highest doses of PCB153 were statistically greater than the number of tumors seen in females rats given only 300 ng/kg-d of PCB126, suggesting that, at doses of PCB153 sufficient to cause extensive induction of microsomal enzymes, there is some additional 'tumor promotion' effects of PCB153 in animals given large doses of PCB126. As with both the individual PCBs, the mixture caused a significant reduction in breast tumors, both malignant adenocarcinoma and benign fibroadenomas.

2. Role of PCBs as liver tumor promoters:

Numerous studies using two stage, 'initiation-promotion' studies have demonstrated that Aroclors act as tumor promoters in the two stage bioassays. For example, Kimura *et al.* (1976) demonstrated that 400 ppm Kanechlor 400 for 6 months increased the incidence of 3'-methyl-4-dimethylaminoazobenzene -initiated liver tumors in female Donryu rats. Nishizumi (1979) showed that Kanechlor 500 increased the incidence of DEN-initiated liver tumors in Wistar rats. Tatematsu *et al.* (1979) showed that Kanechlor 500 developed neoplastic nodules after a non-carcinogenic dose of 2-AAF. Preston *et al.* (1981) showed that 100 ppm of Aroclor 1254 for 18 weeks following initiation with diethylnitrosamine increased the incidence of hepatocellular carcinoma in male Sprague-Dawley rats. And Pereira *et al.* (1982) showed that 500 mg/kg Aroclor 1254 reduced the time to appearance of neoplastic nodules in partially hepatectomized male Sprague-Dawley rats. Ito *et al.* (1973b) showed that PCBs promoted benzene hexachloride induced liver tumors in mice.

One other initiation-promotion study in Sprague-Dawley rats Makiura *et al.* (1974) found that PCBs protected male Sprague-Dawley rats from the tumor-inducing effect of 3'-methyl-4-dimethylaminoazobenzene, N-2-fiorenylacetamide, and diethylnitrosamine. However, in this study the animals only received PCBs for 20 weeks, which was enough to cause significant P450 induction (which likely contributed to enhanced detoxification of the carcinogens) but was apparently not long enough to contribute a promotional effect on later tumor development of initiated foci.

Given that, in every study the doses of PCBs used were high (100 ppm or higher), there is no question that liver microsomal enzymes were greatly induced, including AhR induction of CYP1A1 and many other genes, and CYP2B and 3A induction via CAR and PXR, and that the promotional effect was likely secondary to the extensive physiological changes due to enzyme induction. For example, Preston *et al.* (1981) demonstrated that the promotional effects of Aroclor 1254 that had been 'stripped' of low levels of chlorinated dibenzofurans that are potent activators of the AhR was substantially less effective as a promoter than Aroclor 1254 mixture that contained trace levels of chlorinated dibenzofurans. Since Aroclor 1254 contains significant amounts of PCB126 and PCB118, which are effective activators of the rat AhR, it likely that the promotional effects of Aroclor 1254 are largely, if not fully, mediated via AhR induction. This conclusion is supported by the NTP 2006 study of PCB153, which found no significant tumor development in rats given 100 ppb of PCB153 for 2 years.

3. Summary of 'Time to Tumor' information in these studies:

IBT Studies – no useful 'time to tumor' data were provided in these studies. But there were also no tumors found, even at the end of the study, so these are largely non-informative as to when liver tumors might first appear.

Ito *et al.* (1973b)- These studies, done in mice, involved exposure for 37 weeks, with subsequent sacrifice and histology. The highest dose (500 ppm) of the most highly chlorinated PCB (Kanechlor 500) resulted in 5 of 12 male mice with hepatocellular carcinoma. This is the only study to date that utilized mice.

Kimbrough *et al.* (1975)- There was no useful 'time to tumor' information provided in this study, as apparently no histopathology was performed on animals that died prior to the end of the study. By the survival of these animals was unusually high, as 173 out of 200 control rats, and 184 out of 200 Aroclor 1260-treated (100 ppm) were alive 23 months after initiation of the study. Aroclor-induced tumors did not result in premature mortality, since overall mortality was somewhat lower in the PCB-treated animals (86.5% in control, 92% in Aroclor 1260-treated rats).

4. Summary and conclusions of findings across the various studies:

Table A3-7 summarizes the tumor outcomes for liver tumors in 27 different studies where animals given 100 ppm of Aroclors. Statistically significant increases benign (adenoma) + malignant (carcinoma) liver tumors were seen in 9 of the 27 studies (33%), and carcinomas were seen in 6 of the 2-year rat bioassays of PCB mixtures (Aroclors) at 100 ppm daily dose, as summarized above. Statistically significant increases in tumors were not seen at daily doses of less than 50 ppm in any studies that used lower doses.

Several points are evident from this table (significant effects highlighted in yellow):

1. Studies in which both male and female rats were used found a substantially greater incidence of hepatic tumors in females compared to males. For example, the study by Norback and Weltman (1985) with Aroclor 1260 found 91% liver tumor incidence in female Sprague-Dawley rats, but only one liver tumor (4%) in the 46 treated male rats. Mayes *et al.* (1998) found liver tumors in female Sprague-Dawley rats given 100 ppm Aroclors at the following rates: 1242, 29%; 1254, 55%; 1260, 8%. They found no (1242) or very low (1254, 1260) incidence of liver tumors in male rats. However, the one study with Clophen A60 by (Schaeffer *et al.*, 1984) found a high level of hepatic tumors in male Wistar rats. Whether this unusual effect in males was due to a strain difference or a difference in PCB source is not known.
2. Although malignant tumors (hepatocellular carcinomas and/or cholangiocellular carcinomas) were found in a few animals, the vast majority of tumors seen in both male and female rats were benign adenomas. The exception to this is the Norback and Weltman (1985) study of Aroclor 1260, where they noted that *"Although the tumors met the morphologic criteria for malignancy, their biologic behavior was relatively unaggressive. The neoplasms did not metastasize to distant organs nor invade blood vessels. Mortality of the animals was not increased."* Thus, although they classified them histologically as carcinomas (cancers), the behavior of those tumors was more like benign adenomas than malignant carcinomas, similar to what has been reported in other studies.
3. Liver tumors were much more prevalent in studies on higher chlorinated Aroclor mixtures (e.g., PCB1254 and 1260), than the lower chlorinated mixtures (e.g., Aroclor 1016 and 1242). This is consistent with the relative levels of DL-PCBs in these Aroclors. For example, Rushneck *et al.* (2004) measured all of the DL-PCBs in a variety of Aroclors, and found the following TEQs (using WHO TEF values): 1016, 0.09; 1242, 5.2; 1248, 15; 1254, 21; 1260, 3.5; 1262, 1.1. Thus, 1254 has 6 times the TEQ of 1260, but 1260 had nearly 40 times the TEQ as 1016.

4. The Fisher strain of laboratory rat appears to be much more resistant to Aroclor-induced tumor formation than Sprague-Dawley (SD) rats. This observation has mechanistic relevance, as estrogen production over the lifespan in these two strains is substantially different, with SD rats having prolonged estrous, and thus higher levels of estrogen later in life. This may also explain the high 'background' level of mammary tumors in Sprague-Dawley rats (Brown *et al.*, 2007; Eldridge *et al.*, 1999).

Table A3-7. Summary of tumor incidence from 2 year rat bioassays on various Aroclors at 100 ppm

100 ppm Aroclor 2 yr studies – Excess liver tumors- (controls were subtracted from total observed in treated)								
Study, strain	Aroclor	Months	# males	# females	A+C/total M	C/total M	A+C/total F	C/total F
IBT (1971b), S-D	1254	23-24	35	35	0/11 (0%)	0/11 (0%)	1/14 (7%)	0/14 (0%)
IBT (1971a), S-D	1242	23-24	35	35	0/6 (0%)	0/6 (0%)	0/14 (0%)	0/14 (0%)
IBT (1971c), S-D	1260	23-24	35	35	0/10 (0%)	0/10 (0%)	0/15 (0%)	0/15 (0%)
Kimbrough <i>et al.</i> (1975), Sherman	1260	23	0	184	-	-	26/184 (14%)*	26/184 (14%)*
NCI (1978), F344	1254	24	24	24	3/24 (12.5%)	2/24 (8%)	2/24 (8%)	0/24 (0%)
Schaeffer <i>et al.</i> (1984), Wistar	CloA30	13-23	152	-	Not reported	0/122 (0%)	Not done	Not done
	CloA30	23-27 (died)	152	-	Not reported	1/107 (1%)	Not done	Not done
	CloA30	27+	152	-	Not reported	3/87 (3%)	Not done	Not done
	CloA60	13-23	141	-	Not reported	0/115 (0%)	Not done	Not done
	CloA60	23-27 (died)	141	-	Not reported	9/85 (11%)	Not done	Not done
	CloA60	27+ (all)	141	-	Not reported	52/85 (61%)*	Not done	Not done
Norback and Weltman (1985), S-D	1260	29 ¹	50	50	2/46 ² (4%)	2/46 (4%)	43/47 ³ (91%)*	43/47 (91%)*
Ward (1985), F344	1254	~28	24	24	7/24 (29%)*	2/24 (8%)	2/24 (8%)	0/24 (0%)
Mayes <i>et al.</i> (1998) ⁴ , S-D	1016	24	50	50	0/50 (0%)	0/50 (0%)	6/50 (11%)*	1/50 (2%)
“	1242	24	50	50	0/50 (0%)	0/50 (0%)	15/50 (29%)*	2/50 (4%)
“	1254	24	50	50	2.5/50 (12%)	0/50 (0%)	28/50 (56%)*	6/50 (12%)*
	1260	24	50	50	6.5/50 (12%)	1.5/50 (3%)	24/50 (48%)*	5/50 (10%)*

Footnotes for Table A3-7: (1) Animals received 100 ppm Aroclor 1260 for 16 months, then 50 ppm for 8 more months. Final sacrifice was at 29 mo. Tumor totals appear to include all tumors found, including those sacrificed at interim intervals, and those that received partial hepatectomy; (2) Includes 8 males that received partial hepatectomy during first 18 mo.; (3) Includes 7 females that received partial hepatectomy during first 18 mo. (4) Because there were liver adenomas and carcinomas seen at low frequency in the controls, the % observed in controls was subtracted from the % observed in the 100 ppm treated groups. This series of studies is referred to as ‘Brunner *et al.*, 1997’ in the EPA Risk assessment of PCBs; (5) * (Yellow highlight) emphasizes statistically significant findings.

Based on these studies, EPA and most other regulatory agencies have classified Aroclor mixtures as potentially carcinogenic to humans, and utilized liver tumor incidence in female rats (including benign tumors) in quantitative cancer risk assessments for PCBs (US EPA, 1996). Indeed, virtually all regulatory guidance for environmental PCBs (clean-up standards, emissions standards, effluent standards, etc.) utilizes 'cancer' as the driving end-point (the toxic endpoint with the highest level of risk). The US EPA risk assessment of PCBs, which was completed in 1996, makes three critical assumptions which serve as the basis for all regulatory actions:

- Assumption 1: PCBs are carcinogenic in laboratory rats
- Assumption 2: The slope of the dose-response curve is linear at low doses (below a defined 'point of departure' used to extrapolate high dose rat data to low dose human exposures and response)
- Assumption 3: The liver tumor response seen in female rats at high doses is relevant to humans exposed to much lower doses.

However, although there is clear evidence that PCBs are carcinogenic in some strains of laboratory rats, there is now solid scientific evidence to demonstrate that assumptions 2 and 3 are incorrect.

C. Adverse Outcomes Pathway analysis of AhR activation as the mode of action of PCBs in causing liver cancer in rats

1. Adverse Outcomes Pathways (AOPs) approach for risk assessment of dietary PCBs

In 2010, Ankley *et al.* from the US EPA (Ankley *et al.*, 2010) proposed that the AOP approach should be used for ecological risk assessments that are used for regulatory purposes:

Bringing the full range of emerging tools and understanding to bear on ecological risk assessment requires the development of a framework within which data and knowledge collected at many levels of biological organization can be synthesized in a way that is useful to risk assessors and the ecotoxicologists who support this activity.

This was the first paper to propose using the 'Adverse Outcome Pathway' approach for risk assessment purposes. He provided the following 'flow chart' to conceptualize the process, based primarily on ecological risk assessment (Figure A3-1).

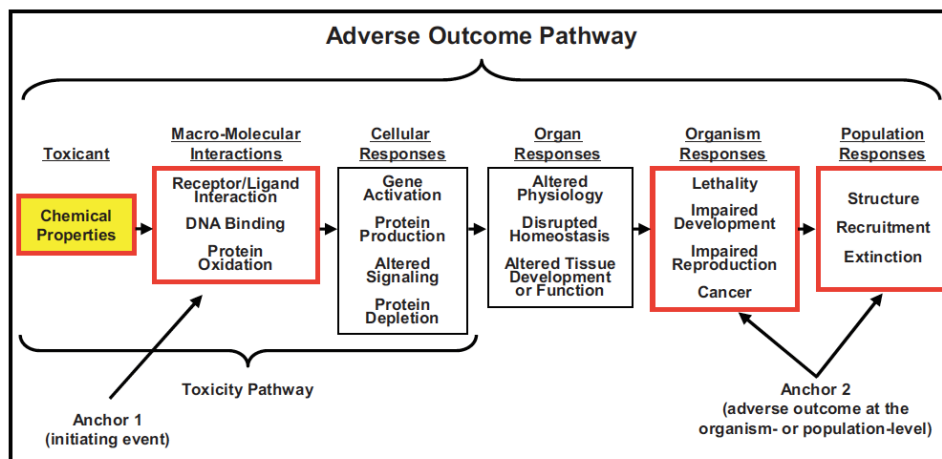


Figure A3-1. Conceptual diagram of key features of an adverse outcome pathway (AOP). Each AOP begins with a molecular initiating event in which a chemical interacts with a biological target (anchor 1) leading to a sequential series of higher order effects to produce an adverse outcome with direct relevance to a given risk assessment context (e.g., survival, development, reproduction, etc.; anchor 2). The first three boxes are the parameters that define a toxicity pathway, as described by the National Research Council (figure from: Ankley *et al.*, 2010)

A recent workshop sponsored by NIEHS, EPA and other environmental science/regulatory agencies summarized the current scientific thinking about how AOPs will improve the quality and reliability of chemical risk assessments for regulatory purposes. The Workshop summary Kleinstreuer *et al.* (2016), states the following:

Models for toxicity testing are evolving from traditional observational models to models based on improved understanding of toxicity mechanisms. This new approach will enable the use of predictive tests and models to better inform regulatory decisions in a more timely fashion. One element of this paradigm shift is the concept of the adverse outcome pathway (AOP). The AOP is a conceptual framework constructed from existing knowledge relating toxic substance exposures to subsequent molecular and cellular changes resulting in illness or injury to an individual or population (Ankley et al., 2010). AOPs are made up of specific biological elements, which may be shared between AOPs and act as connecting nodes for AOP networks:

- *A molecular initiating event (MIE) is an interaction, such as a chemical binding to a receptor or to proteins, which must occur to begin the toxicity process.*
- *The MIE is the first in a series of key events (KEs) that must occur for toxicity to progress. Early KEs at the cellular level can include altered protein production or molecular signaling; later KEs can include changes in tissue or organ function. The links between KEs are described by key event relationships (KERs).*

- *Adverse outcomes (AO) are the result of the molecular initiating event and key events. They include diseases, developmental defects, impaired reproduction, etc. Adverse outcomes may be described at the individual or population level, especially when considering environmental effects, where these outcomes might include changes in population structure or local extinction of a species.*

A very similar approach to the AOP is referred to as 'Mode of Action' (MOA), which has been in use in risk assessment of specific chemicals for more than a decade. The two approaches are almost identical, but the AOP workshop (Kleinstreuer *et al.*, 2016) defined the modest difference as follows:

*The concepts of MoA and AOP are very similar. However, an important difference is that a MoA pathway tends to describe details specific to a particular chemical or chemical class, while AOPs describe perturbed biological pathways and are, therefore, ideally, chemical-agnostic. Also, information on events occurring before the MIE, such as metabolism and kinetics, is, when possible, intended to be considered independently from an AOP (DL Villeneuve *et al.*, 2014b). Key shared principles for MoAs and AOPs are that (1) the pathway is a plausible hypothesis of important events, rather than a detailed molecular understanding of each step, and (2) every step in the process or pathway need not be defined in order for the pathway to have utility.*

A recent review on the use of AOPs for regulatory decision-making (Carusi *et al.*, 2018) provides the following description of the AOP approach:

The goal of the AOP framework is to compile and synthesize this wealth of biological information such that it can be transparently and efficiently employed for decision-making. Fig. 2 provides an overview of the AOP framework in the context of its potential application to the translation and use of different types of data to support assessment of the effects of chemicals on human health and the environment. The initial interaction of a chemical with a biological system is depicted as the molecular initiating event (MIE), such as binding to a protein (e.g., receptors, enzymes) or DNA, or interactions with membrane lipids. These MIEs can cause subsequent perturbations at higher biological levels of organization, depicted as intermediate key events (KEs) along an AOP, which ultimately may result in adverse apical responses such effects on survival, reproduction, carcinogenesis, etc.

This approach is useful for both human health and ecological risk assessments. The focus of this report will be to use AOPs to assess the potential adverse health outcomes to humans exposed to PCBs via the diet. The concept behind the AOP approach is that the potential adverse outcomes associated with a specific biological pathway are dependent upon the Molecular Initiating Event (MIE), which is the ability of the toxic substance to initiate a key molecular event that is necessary, but usually not sufficient by itself, to cause an adverse effect. For example, certain dioxins, dibenzofurans and co-planar PCBs can bind to and activate the aryl hydrocarbon receptor (AhR) with varying degrees of affinity (potency) and efficacy (magnitude). In this example of an AOP, activation of the AhR by its cognate ligand (Dioxins, DL-PCBs, etc.) is the Molecular Initiating Event (MIE, or Key Initiating Event, KIE) (Figure A3-2).

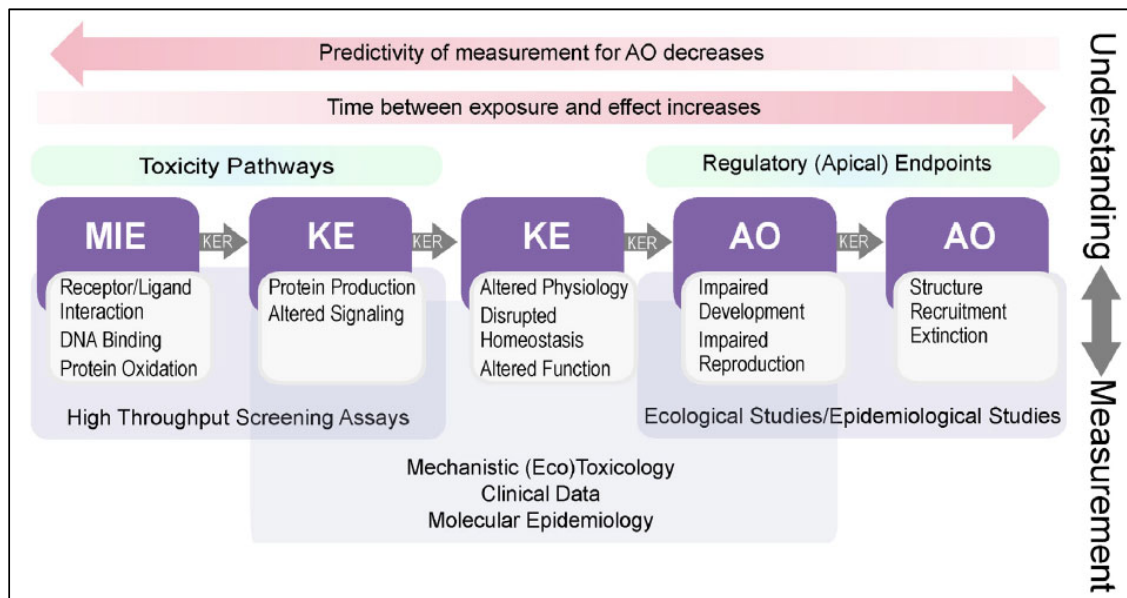


Figure A3-2. Depiction of the role of the Adverse Outcome Pathway (AOP) framework in linking various data streams to outcomes relevant to regulatory decision-making for chemicals. MIE –molecular initiating event, KE – key event, KER – key event relationship, AO – adverse outcome. Source: from: Ankley and Edwards (2018), as published in Carusi *et al.* (2018)

The 2016 EPA/NIEHS AOP Workshop (Kleinstreuer *et al.*, 2016) noted the following about the utility of AOPs for regulatory decision making in toxicology:

An AOP is a framework that allows the placement of available information on biological pathways into an organized, usable, testable format. As outlined above, information in an AOP could be used for assessing chemical risks in a number of ways, including prioritization of chemicals for future evaluation, development of predictive models and IATAs, qualitative or quantitative hazard characterization, and ultimately risk assessment. The utility of an AOP depends on the completeness and maturity of the knowledge underpinning the AOP, the extent to which the links between each KE are understood, and how easily the KEs can be queried.

The Workshop also provided the following perspectives on implementation and value of using an AOP approach to quantitative risk assessment:

At its most basic level, a useful AOP links a MIE convincingly and qualitatively to an [adverse outcome] AO. Adding information about the linkages between the intermediate KEs along a pathway expands the usefulness further, perhaps to the level where evidence provided by mechanistic tests querying particular KEs can characterize chemical hazards. However, for maximal utility, these linkages, or KERs, must be understood quantitatively. Beyond characterizing the pathway, it is important to understand the dose that

activates the pathway, and if it is relevant to human or ecological exposure scenarios [emphasis added].

Thus, this expert report provides an AOP approach to assessing the adverse outcomes that may result from dietary exposures to PCBs from consumption of fish that have bioaccumulated PCBs from their environment.

The process for conducting an AOP-focused, site-specific risk assessment for potential health effects of consumption of fish contaminated with PCBs involves the following steps/questions:

1. What is the 'key molecular initiating event' (MIE, that leads to a Key Initiating Event, KIE, that is a biological response, such as changes in gene expression) for the specific health outcome of interest (e.g., cancers, reproductive effects, immune toxicity, neurotoxicity, etc.) identified in toxicological studies in experimental animals?
2. What is the shape of the 'dose-response' (D-R) curve for the KIE (or for a biomarker that reflects the KIE)?
3. For complex mixtures such as PCBs, what is the D-R relationship for the specific chemicals/congeners of interest that operate through the KIE?
4. From D-R analysis of experimental animal data from chronic or sub-chronic *in vivo* bioassays, what is the daily dose of specific chemicals/congeners of interest necessary to produce a biologically significant response through the KIE? (e.g., in the case of AhR activation as the KIE, what daily dose, in TEQ, of AhR-activating ligands is necessary to cause an increase in AhR-response that is 20% of the maximal response at steady-state, i.e., a Benchmark Concentration 20%, BMC20)?
5. If the daily dose necessary to obtain a biologically significant response is obtained from experimental animal studies, what is an evidence-based 'species correction factor' that can reasonably adjust for species differences in the dose-response relationship between experimental species used (e.g., rats, mice, monkeys) and humans?
6. Once a human estimate of daily dose of the biologically significant response is obtained, identify from the D-R analysis a further adjustment to identify a daily dose that will have 'no biological effect' (e.g., a threshold response for the KIE). This will vary depending on the slope of the D-R for the KIE, but will likely be somewhere between a factor of 2 and a factor of 10 lower than the BMC20.
7. If, as is the case with certain PCB congeners of interest, the specific chemical(s)/congener(s) of interest are not actually measured in exposed human populations, is there a means to accurately estimate the dose of the congener of interest from a measurement of other chemicals/congeners? (e.g., can one reasonably estimate the concentration of PCB126 from a measurement of total PCBs, where PCB126 was not one of the specific analytes?)
8. Once a reasonable estimate of the daily dose, in TEQ, of chemicals/congeners of interest that would result in no significant biological response of the KIE (KIE threshold) is established, comparisons of this dose to estimates of the dose of chemical/congeners of interest in exposed populations can be determined and 'Margins of Exposure' (MOE) established. (MOEs reflect the ratio of the 'threshold' blood concentration to the concentration measured or estimated to occur from specific exposure scenarios).

Answering the 8 questions above will provide an evidence-based approach to estimating the daily dose of total PCBs in edible tissues of fish that could reasonably be anticipated to cause some measurable biological response (e.g., sufficient to activate the Key Molecular Initiating Event to produce a measurable biological response, or KIE) in humans, for each type of adverse response. From that, one can then estimate the 'Margins of Exposure' that exist under different conditions of exposure (fish concentration and fish consumption rate).

2. Activation of the Aryl Hydrocarbon Receptor (AhR) as a primary Adverse Outcomes Pathway (AOP) for the toxicological effects of Dioxin-Like PCBs

As discussed in the main body of this report, it is widely recognized that the toxicological effects of the DL-PCBs are dependent upon their relative abilities to bind to and activate the AhR (the Molecular Initiating Event in an AOP approach). Based on this widely recognized concept, every regulatory body in the world with authority for PCB regulations utilizes the concept of 'Toxic Equivalency Factors', or TEFs, to assess the potential toxicity of PCB mixtures. Another term, referred to as 'Relative Effective Potency', or REP, is essentially identical to the TEF approach. TEFs are the 'correction value' derived from experiments that calculate a REP. The ability of individual dioxins, dibenzofurans and dioxin-like PCB congeners is compared to the effect of TCDD in activating the AhR. Thus, REPs are potency estimates relative to TCDD, which is set to a value of 1. Thus, a compound that was 10% as potent as TCDD in activating the AhR would have a REP, or TEF value of 0.1. Since the early 1990s, the World Health Organization (WHO) has organized expert meetings to review the scientific basis for establishing the toxic equivalency factors (TEFs) for dioxin and dioxin-like compounds at the international level, thus providing consistent approaches to risk assessment to national regulatory authorities (van den Berg *et al.*, 2006).

Numerous recent reviews have exhaustively described the sequence of events that occur following extensive activation of the AhR by dioxins and dioxin-like compounds. The complex series of 'downstream' events that lead to changes in gene expression and associated biochemical perturbations result in a wide variety of adverse effects, including but not limited to tumors, immune toxicity, liver toxicity, chloracne and other dermatological effects, and adverse reproductive and development outcomes. Thus, all of these adverse outcomes are dependent upon the 'Key Initiating [Molecular] Event' of activation of the AhR. If the dose/concentration of the activating ligand is insufficient to activate the AhR, then there will be no 'downstream events' and no toxicity. Therefore, to estimate the potential human health risks from exposures to 'DL-PCBs' it is only necessary to identify the dose-response relationship for the DL-PCBs that can activate the human AhR, and determine a biological 'no effect' level in target tissues or associated steady-state blood concentrations (e.g., identify the maximum exposure level of an AhR ligand such as PCB126 that will not have any significant effect on the human AhR. Exposure levels below this 'benchmark dose' will then have no toxicological effects via the AhR MOA/AOP.) Once a 'Point of Departure' daily dose from the animal studies (i.e., the 'threshold' dose, in units of ng of TCDD equivalents /kg bw/day) is identified, it is then possible to estimate 'Margins of Exposure', or MOEs, under specific exposure scenarios (e.g., a given concentration of DL-PCBs/TEQs in fish, and a given rate of fish consumption). Human daily dose is adjusted by species differences and scaling factors for using rat data to identify the human equivalent 'threshold' dose. MOEs of greater than 100 would generally be viewed as 'safe.'

The most convenient way to go about assessing dose-response functions for AOPS is to do so by specific adverse outcomes. Thus, this report will first evaluate the AOP for liver cancer induced by dioxins and DL-PCBs in animal studies.

3. Using an AOP/MOA approach to assess the potential CARCINOGENIC risk of PCB exposures via long term consumption of fish containing PCBs

In 2012, an international workshop was held to conduct a comprehensive evaluation of AhR activation as the mode of action of liver tumors seen in rats following administration of dioxins and dioxin-like compounds. The report from the workshop was published in 2014 (Budinsky *et al.*, 2014). In the report, the rationale for this workshop was described as follows:

“Despite extensive knowledge of the toxicology of dioxins, no mode of action (MOA) hypothesis for their tumorigenicity has been formally documented using the Human Relevance MOA framework developed by the International Programme on Chemical Safety (IPCS). To address this information gap, an expert panel was convened as part of a workshop on receptor-mediated liver tumorigenicity.” The introduction to the report provides an excellent description of the value and rationale for using a MOA/AOP approach for risk assessment of the putative carcinogenic actions of dioxins and dioxin-like compounds: “The aryl hydrocarbon receptor (AHR) pathway is one of the most studied in toxicology. It mediates the biological activity of dioxin-like chemicals that include 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), the most potent AHR agonist, as well as several less potent dioxin-like chemicals, including 6 other polychlorinated dibenzo-p-dioxins (PCDDs), 10 polychlorinated dibenzofurans (PCDFs) and 12 coplanar polychlorinated biphenyl (PCBs) congeners. Controversy has swirled around the carcinogenicity of dioxin-like chemicals in rodents regarding whether the dose–response is best understood as a linear non-threshold or a non-linear threshold phenomenon, and, not least, the human relevance of this rodent response (JEFCA, 2003; US EPA, 2010).”

The Budinsky *et al.* (2014) ‘AhR’-focused report, in addition to the more recent reviews of the AOP approach discussed previously, provide an excellent foundation for using a MOA/AOP approach to conduct site-specific risk assessments for potential **carcinogenic** risk of DL-PCBs in humans exposed via fish consumption. The Budinsky *et al.* (2014) report provided an excellent MOA/AOP diagram showing the critical steps (including the Key Initiating Event of ‘sustained AHR Activation’) that leads to the formation of liver tumors in rats exposed to dioxins and dioxin-like compounds (Figure A3-3).

The members of the AhR panel provided the following summary statement regarding chemicals that activate the AhR and increase tumors in laboratory animals:

The AHR panel members concluded that TCDD acts via a tumor promotion MOA with sustained near maximal AHR activation for a significant portion of the life span as the pivotal and initial KE; the apical outcome of this MOA is the late development of female rat liver tumors. At the cell and tissue levels, the other two KEs were: (1) altered focal cell proliferation (i.e. changes in cell division and apoptosis) and (2) pre-neoplastic focal tissue changes (i.e. hyperplasia and other histopathological observations).”

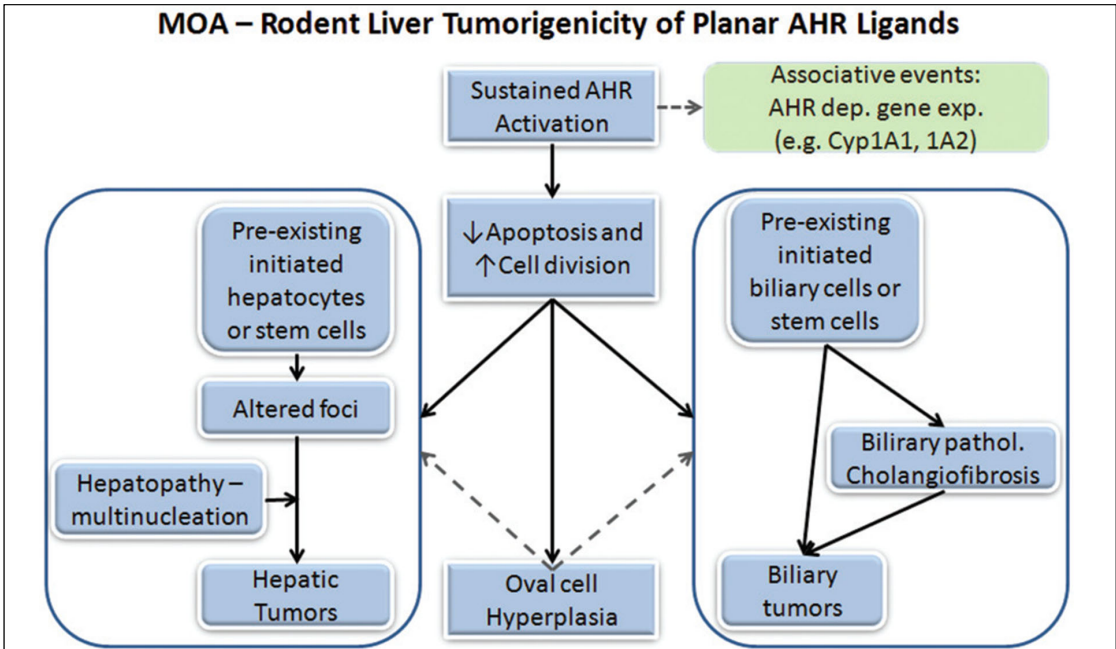


Figure A3-3. Proposed schematic of the MOA hypotheses developed by the AHR Case Study Panel

“Postulated mode of action with key events of rodent liver tumors induced by AHR agonists. Sustained activation of the AHR regulates the transcription of different classes of genes including those involved in cell proliferation and apoptosis. AHR-induced changes in gene transcription occur within altered cells of either a biliary or hepatocellular lineage to expand and promote the eventual development of cholangio- and hepatocellular adenomas and carcinomas. Inhibition of intrafocal apoptosis also facilitates the survival of initiated cells that would otherwise undergo apoptosis. Central to this tumor promotion scheme is the role of oval cell (stem cell) proliferation with potential impacts on normal differentiation. Histopathological changes noted in the descriptor “hepatopathy”, e.g. multinucleated hepatocytes, further contribute to the expansion of pre-neoplastic. At higher-doses, elements of necrosis and regenerative repair may serve to increase cell proliferation.” (from: Budinsky *et al.*, 2014).

Mode of Action assessment for liver cancer in rats from PCBs

An extensive biochemical and molecular analysis by Brown *et al.* (2007) of the Sprague-Dawley (SD) rats used in the Mayes *et al.* (1998) bioassay of the multiple Aroclors provides a compelling demonstration of the mode of action of PCB mixtures in inducing hepatic tumors in both male and female SD rats, and also explains both the remarkable sex and species differences in incidence of Aroclor-induced liver tumors in rats seen across the multiple different studies. By measuring the levels of different CYP enzyme activities and mRNA levels, as well as a host of molecular/ biochemical ‘intermediate biomarkers’, such as superoxide radical formation and estrogen metabolites in the various groups of rats used in the Mayes *et al.* (1998) studies (different sex, different dose, those with and without tumors, etc.), the authors were able to construct a thorough MOA for PCB-induced liver tumors in female SD rats. Table A3-8 below shows the tumor formation incidence in both males and females, at different Aroclor mixtures and dose levels of those mixtures.

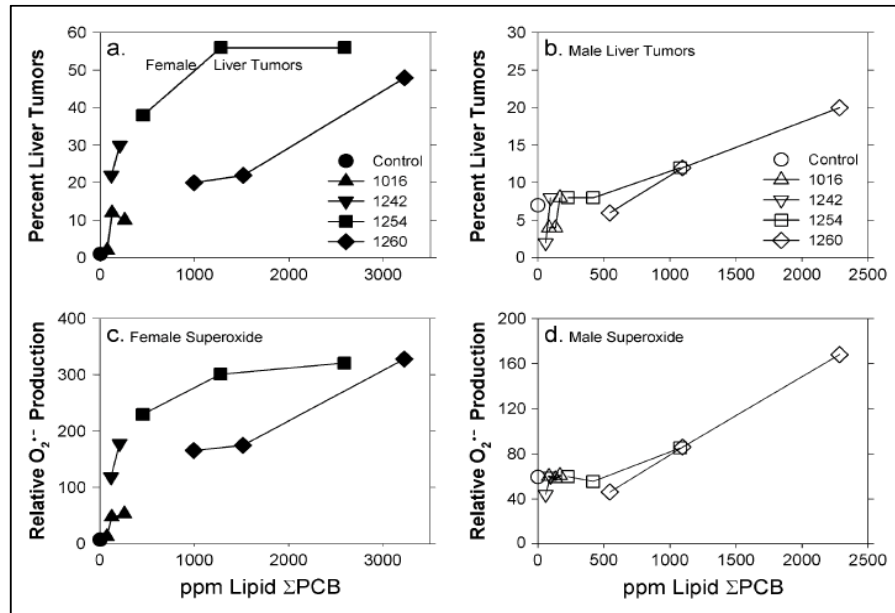
Table A3-8. Incidence of Neoplastic Liver Lesions (from: Mayes *et al.*, 1998)

Group	Dose (ppm)	Sex	Hepatocellular adenoma (%)	Hepatocellular carcinoma (%)	Hepatocholangioma (%)	Hepatocholangiocarcinoma (%)	First tumor ^b	Total animals with neoplasms (%)
Control ^a	0	M	4 (4)	3 (3)	0 (0)	0 (0)	91	7 (7)
Aroclor 1016	50	M	1 (2)	1 (2)	0 (0)	0 (0)	105	2 (4)
	100	M	1 (2)	1 (2)	0 (0)	0 (0)	105	2 (4)
	200	M	2 (4)	2 (4)	0 (0)	0 (0)	79	4 (8)
Aroclor 1242	50	M	1 (2)	1 (2)	0 (0)	0 (0)	68	1 (2)
	100	M	3 (6)	1 (2)	0 (0)	0 (0)	85	4 (8)
Aroclor 1254	25	M	2 (4)	2 (4)	0 (0)	0 (0)	87	4 (8)
	50	M	2 (4)	2 (4)	0 (0)	0 (0)	88	4 (8)
	100	M	6 (12)	0 (0)	0 (0)	0 (0)	92	6 (12)
Aroclor 1260	25	M	2 (4)	1 (2)	0 (0)	0 (0)	96	3 (6)
	50	M	5 (10)	1 (2)	0 (0)	0 (0)	86	6 (12)
	100	M	7 (14) ^c	3 (6)	2 (4)	0 (0)	50	10 (20) ^c
Control	0	F	1 (1)	0 (0)	0 (0)	0 (0)	105	1 (1)
Aroclor 1016	50	F	1 (2)	0 (0)	0 (0)	0 (0)	105	1 (2)
	100	F	5 (10) ^c	1 (2)	0 (0)	0 (0)	87	6 (12) ^d
	200	F	5 (10) ^c	0 (0)	0 (0)	0 (0)	63	5 (10) ^c
Aroclor 1242	50	F	10 (20) ^d	0 (0)	1 (2)	1 (2)	72	11 (22) ^d
	100	F	12 (24) ^d	2 (4)	2 (4)	0 (0)	87	15 (30) ^d
Aroclor 1254	25	F	18 (36) ^d	0 (0)	2 (4)	0 (0)	86	19 (38) ^d
	50	F	26 (52) ^d	4 (8) ^c	6 (12) ^d	0 (0)	72	28 (56) ^d
	100	F	27 (54) ^d	6 (12) ^d	1 (2)	0 (0)	82	28 (56) ^d
Aroclor 1260	25	F	9 (18) ^d	1 (2)	0 (0)	0 (0)	76	10 (20) ^d
	50	F	10 (20) ^d	1 (2)	0 (0)	0 (0)	84	11 (22) ^d
	100	F	21 (42) ^d	5 (10) ^d	3 (6) ^c	0 (0)	69	24 (48) ^d

^a *n* = 100 for control, all other groups *n* = 50.^b Study week of first occurrence.^c Statistically significantly different from control at *p* ≤ 0.05 (Fisher's exact test).^d Statistically significantly different from control at *p* ≤ 0.01 (Fisher's exact test).

There were two analyses of particular importance in elucidating the MOA for liver tumors in male and female SD rats exposed to Aroclor 1242, 1254 and 1260 (no tumors were seen in either male or female SD rats exposed to Aroclor 1016). First, and not surprisingly, the tumor incidence was highly correlated with body burden of total PCBs. For female rats, the tumor incidence increased quickly in a dose-related fashion, and for Aroclor 1254 seemed to plateau at a total PCB concentration of >1000 ppm in body fat (lipid). Tumor incidence in males was much lower, and was dose related at the highest body burden levels (no increase in tumors above the background of 4% was seen at body burdens of less than 1000 ppm in body fat, but was approximately 10% and 20% at body burden levels of 1250 and 2500 ppm lipid, respectively; Figure A3-4).

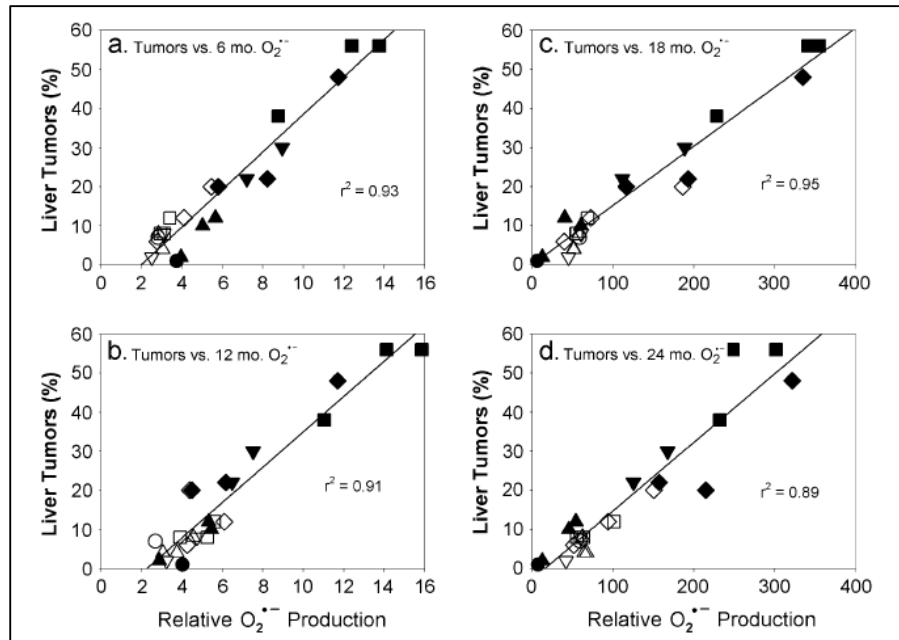
Figure A3-4.
Dependency upon mean midlife (6-18 months) adipose SumPCB, Aroclor type, and rat sex for (a,b) incidence of liver tumors of any type after 24 months continuous Aroclor dosing; and (c, d) production of superoxide ($O_3^{\bullet-}$) equivalents by reoxidized test rat liver cytosols when exposed to NADPH, O_2 and



Phenobarbital-induced control rat microsomes (from: Brown *et al.*, 2007)

However, in addition to measuring PCB cumulative body burden in fat, the authors also measured the production of reactive oxygen species in the liver, in the form of superoxide anion ($O_3^{\bullet-}$). They found that the production of $O_3^{\bullet-}$ was highly correlated with the body fat accumulation of total PCBs (Figure A3-4). They further examined the correlation between liver tumors in both male and female rats, and found a remarkably strong correlation ($r^2 = 0.9$ or greater) in both males and females between the level of production of $O_3^{\bullet-}$ and tumor incidence. However, there was a dramatic difference in the absolute levels of $O_3^{\bullet-}$ between males and females, with females having much higher levels of both tumors and $O_3^{\bullet-}$ (Figure A3-5).

Figure A3-5.
Relationship of % rats with any liver tumor versus cytosol-catalyzed production of superoxide ($O_2^{\bullet-}$) equivalents at (a) 6, (b) 12, (c) 18, or (d) 24 months as measured using two different control rat microsome preparations. Aroclor symbols are the same as designated in the



previous figure. Open symbols represent males, close symbols, females (from: Brown *et al.*, 2007)

Detailed analyses of estrogen metabolism in these animals demonstrated that high dose PCBs exposures greatly enhanced the oxidation of estrogen to redox-cycling catechol estrogens, which were then largely (but not completely) responsible for the increase in $O_2^{\bullet-}$ / oxidative stress. This explains in large part, if not completely, why tumor incidence is much higher in female than male rats treated with higher chlorinated PCBs- females have much higher circulating levels of estrogen throughout life, and thus life-long exposure of the liver to $O_2^{\bullet-}$ / oxidative stress.

But how do higher chlorinated PCBs increase the formation of estrogen-related $O_2^{\bullet-}$ / oxidative stress in the liver? This was thoroughly addressed in the Brown *et al.* (2007) publication through a careful analysis of the induction levels of various cytochrome P450 (CYP) enzymes that are involved in oxidative metabolism of multiple exogenous and endogenous substances, including estrogen. It has been known since the early 1970s that Aroclor mixtures induce the expression of multiple cytochromes P450, particularly CYP1A1 and CYP1A2, mediated via the AhR receptor, and CYP2B and 3A enzymes mediated largely via CAR and PXR nuclear receptors.

As discussed previously, the Dioxin-like PCBs (non-ortho and mono-ortho substituted) have varying affinities for the AhR, and thus are capable of inducing the expression of CYP1A1, CYP1A2 and CYP1B1 via ligand activation of the AhR.

It is also known that CYP1A enzymes oxidize estradiol, including oxidation to redox-cycling catechol estrogens (Niwa *et al.*, 2015). In the absence of CYP1A induction, most estradiol is largely metabolized to non-catechol oxidation products, which are quickly conjugated and eliminated, and therefore do not induce oxidative stress in the liver. However, induction of CYP1A enzymes via AhR shifts the metabolism toward the formation of more redox-cycling quinones (RCQ = catechol estrogens), producing continuous oxidative stress

in the livers of female rats, especially those that have continuous lifetime circulating levels of estrogen (e.g., SD rats, relative to Fisher rats).

Brown *et al.* (2007) summarize this elegant MOA explanation for both sex and species differences in liver tumor formation in rats exposed to Aroclor mixtures capable of inducing CYPs (1242 [weak inducer], 1248, 1254 and 1260, but not 1016) as follows (Figure A3-6):

1. Total PCB/TEQ accumulation in rat tissues (particularly liver);
2. Total PCB/TEQ repression of constitutive MFOs;
3. Total PCB/TEQ induction of other MFOs, particular AhR-mediated CYP1A genes;
4. MFO-mediated formation of RCQs (estrogen derived redox-cycling catechols/quinones);
5. RCQ-mediated formation of superoxide anion ($O_3^{\bullet-}$; ROS [reactive oxygen species]),
6. ROS dismutation to hydrogen peroxide (H_2O_2); and
7. H_2O_2 -mediated mitotic signaling, resulting in the proliferation of spontaneously or otherwise initiated cells to form hepatic tumors, as in tumor promotion.

The significance of this, in the context of AOPs, is that activation of the AhR via Dioxin-Like PCBs is the Key Initiating Event that ultimately leads to oxidation of estrogen and other CYP1A substrates to redox-cycling quinones (RCQs), which are the ultimate tumorigenic molecules. If the concentration of DL-PCBs in the target tissue (liver) is insufficient to cause significant and sustain activation of the AhR, none of these events will occur. Thus, these studies provide strong evidence that the liver tumors found in multiple 2-year rodent bioassays with various mixtures of higher chlorinated Aroclors are the result of AhR-mediated induction of CYP1A, and perhaps to a lesser extent, PXR and/or CAR-mediated induction of other hepatic CYPs, which then oxidize estrogen and other endogenous molecules to ROS that ultimately promote tumor formation from background-initiated cells.

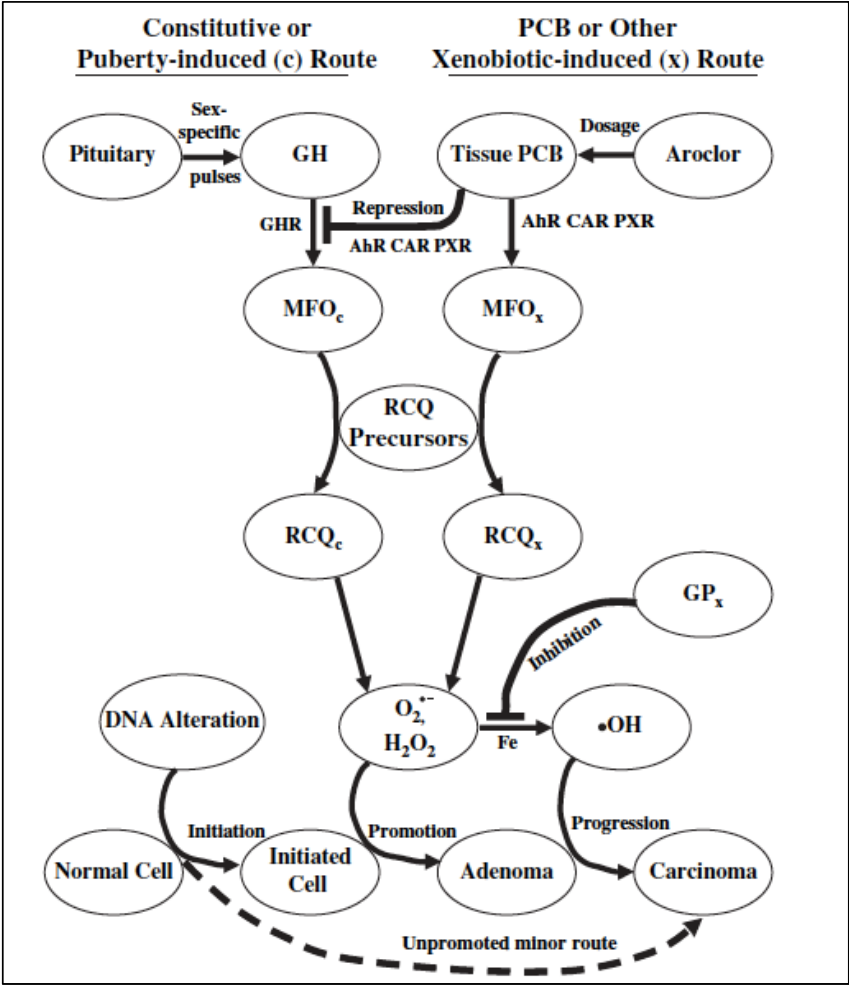


Figure A3-6. Proposed MFO_RCQ_ROS-mediated signaling cascades for tumorigenesis in Aroclor-dosed and undosed S-D rats. Constitutive and/or GH-induced pathway on the left is suppressed by sumPCB/TEQ accumulations, probably via receptor-mediated nuclear receptor-factor degradation. Inducible pathway on the right is stimulated by sumPCB/TEQ accumulations, probably via binding to same nuclear receptor as that which mediates suppression of the constitutive pathway. Both pathways produce tumorigenic H_2O_2 via MFO-RCQ-ROS sequences (from: Brown *et al.*, 2007)

This MOA for rat liver tumors was also discussed in detail in the Consensus Workshop report on the MOA for liver tumors from TCDD exposure (Budinsky *et al.*, 2014). The report provided a summary figure and conclusions about the mode of action of TCDD in inducing liver tumors in rats (Figure A3-7, from Budinsky *et al.* (2014):

The workshop and the AHR panel were able to establish a MOA with defined KEs [Key Events], AEs [Adverse Effects] and ModFs [Modifying Factors] [Figure A3-7]. This MOA can be used not only in risk assessment efforts for dioxin-like chemicals, but also to examine dose-response modeling. With knowledge of the events in the MOA and the range of

doses or tissue concentrations associated with each event, we will be better able to determine the overall dose–response range corresponding to a defined risk level for liver tumor promotion.

Building from the analyses developed at the meeting, a variety of quantitative dose–response assessments for KEs and AEs [within the tumor promotion MOA of TCDD can be described. As the MOA progresses toward tumors, these events represent increasingly complex biological changes.

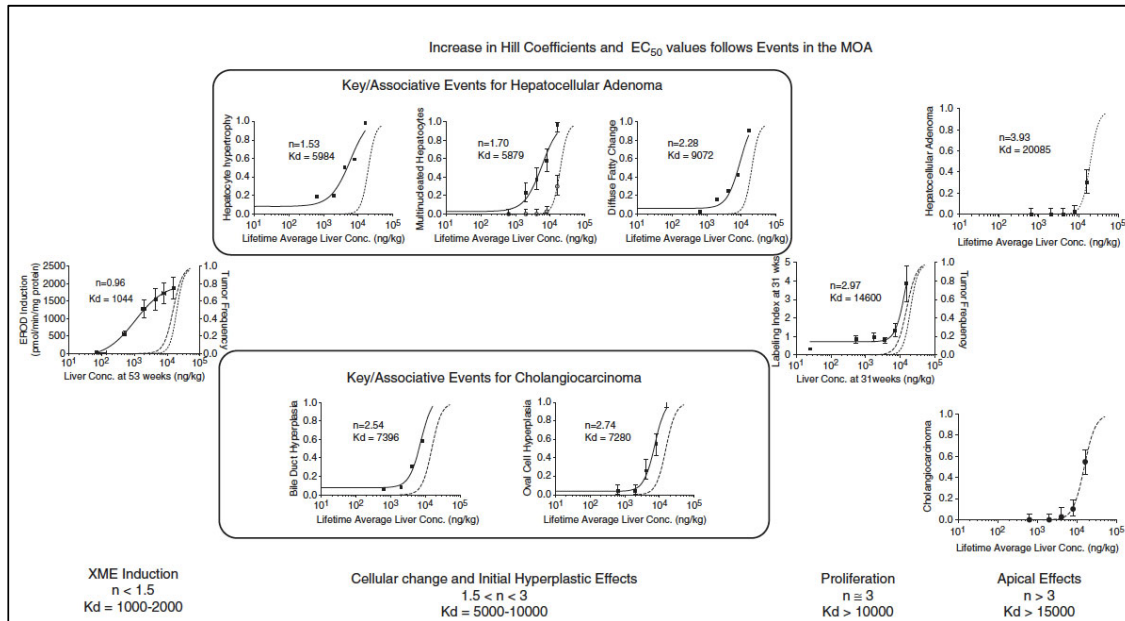


Figure A3-7. Mode of action of TCDD-induced liver cancer in rats using lifetime average liver concentration (LALC) as the dose (apical endpoint curves (dashed and dotted lines) are overlaid on each non-apical endpoint plot (solid lines) to provide a convenient reference for comparison; for EROD at 53 weeks and labeling index at 31 weeks, a right y-axis is included for clarity). The first KE, sustained AHR activation (XME induction; xenobiotic metabolizing enzyme), is measured by dose-dependent induction of EROD, representing the enzymatic activity of CYP1A1. This early low dose–response would likely be linear at low dose because the Hill coefficient (n) is <1.5 (see text for details). As later events in the MOA occur, both the Hill coefficients and the half-maximal concentrations (Kd) increase. Cell proliferation is a seemingly late high-dose event; its Hill coefficient is ~3 and the half-maximal value is 410 000 ng/kg. For the two apical events, hepatocellular adenoma and cholangiocarcinoma, the Hill coefficients are >3 and the half-maximal values are 415 000 ng/kg. This suggests that the apical endpoints are non-linear phenomena and that their occurrence is associated with high doses relative to earlier KEs. (Fig. 4 from: Budinsky *et al.*, 2014)

The Consensus Workshop was focused on liver tumors in rats, as this has been the ‘driver’ of most risk assessments for dioxins and dioxin-like compounds, including PCBs. The workshop proposed the following scheme for risk assessment of dioxins and dioxin-like compounds, including PCBs (Figure A3-8):

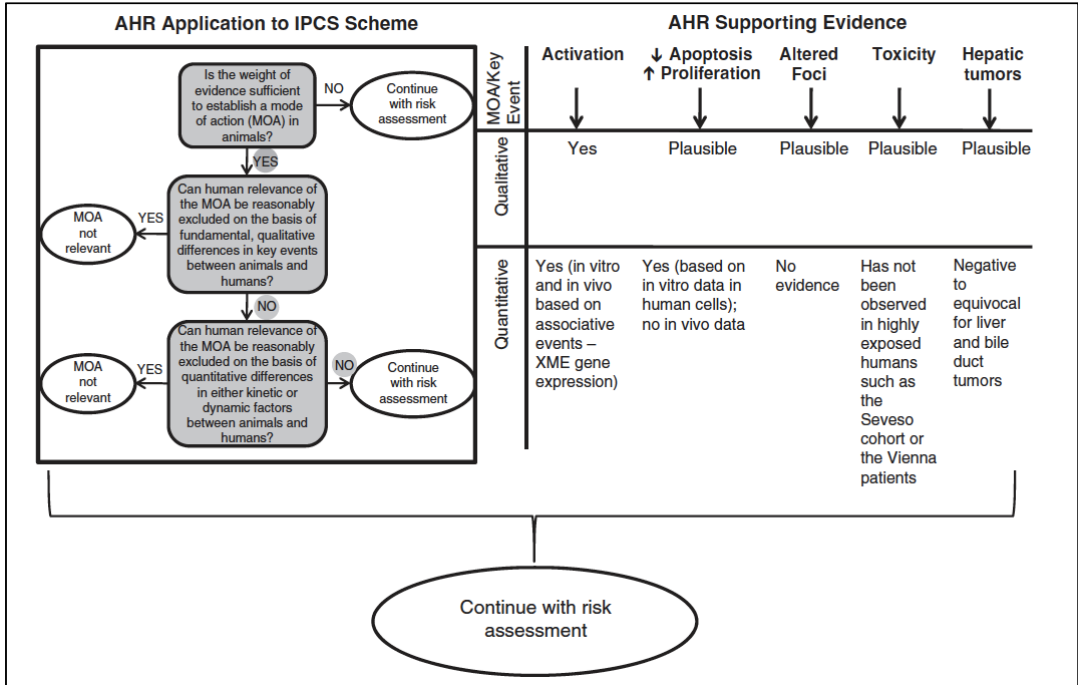


Figure A3-8. Application of the IPCS (International Programme on Chemical Safety) human relevance framework to the hypothesized AhR MOA. The adverse outcome and the key events closer to the apical outcomes (Hepatocellular and cholangiolar cancers) have not been proven to occur in excess or occur at all, respectively, in humans exposed to relatively high levels of TCDD. However the fundamental initial key event of AhR activation has been established to occur in humans following TCDD Exposure resulting in CYP1A induction and chloracne (Fig. 6 from: Budinsky *et al.*, 2014)

As a final point in discussing the evidence that liver tumors in rats following the administration of dioxin-like compounds requires activation of the AhR, Harrill *et al.* (2016) evaluated hepatic changes in Sprague-Dawley rats for which the AhR had been ‘knocked out.’ New genomics technologies now allow scientists to selective remove, or ‘knock out’, specific genes in mouse and rat genomes. In this report, Harrill *et al.* (2016) investigated the role of AhR in mediating pathological changes in the liver prior to tumor formation following 4-weeks of repeated-dosing with 0, 3, 22, 100, 300 and 1000 ng /kg/day TCDD, using adult female wild-type (WT) and AhR knockout (AHR-KO) animals. As expected, they found treatment-related increases in the severity of liver and thymus pathology in the WT rats containing a functional AhR, but no evidence of any pathology in the AHR-KO rats. In the livers of rats with a functional AhR (WT) they observed hepatocellular hypertrophy, bile duct hyperplasia, multinucleated hepatocytes and inflammatory cell foci, but no effects were seen in the rats that lacked a functional AhR (Ahr-KO). A loss of cellularity in the thymic cortex and thymic atrophy was observed in WT. Treatment-related changes in serum chemistry parameters were also observed in WT, but not Ahr-KO rats. Finally, they reported dose-dependent accumulation of TCDD primarily in the liver of WT rats and primarily in the adipose tissue of Ahr-KO rats. These results further demonstrate that AhR activation is the initial key event underlying the progression of histological effects leading to liver tumorigenesis following TCDD treatment.

Dose-Response Analysis of NTP bioassays of PCBs and specific PCB congeners

Based on the compelling evidence from the previous rodent studies demonstrating that mixtures of PCBs can cause liver cancer in rats, but that lower chlorinated mixtures largely devoid of DL-PCBs seemed not to be carcinogenic, the National Toxicology Program (NTP) designed a large number of 2 year rat bioassays to explicitly test the hypothesis that the carcinogenic effects observed in higher chlorinated PCB mixtures bioassays was solely due to the presence of DL-PCBs at sufficient concentration to fully activate the AhR.

Table A3-9. NTP carcinogenesis studies on specific congeners and dioxins

Study	Congener	Dose	EROD 53 wks fold [^]	Cell Proliferation 31 wks (fold [^]) N=10	Liver hepatocyte Tumors, A; [C]	Liver Carcinomas (cholangio)
NTP (2006c)	126	0 ng/kg	1.0	1.0	2%	0%
		10	8*	1.23	-	-
		30	26*	1.68	4%	0%
		100	38*	0.99	2%	2%
		175	41*	1.33	0%	0%
		300	59*	1.62*	4%	9%
		550	49*	1.83*	8%	12%*
		1000	53*	1.78*	13%*	42%*
NTP (2010)	118	0 µg/kg	1.0	1.0	0	0
		30	1.52*	0.73	-	-
		100	4.63*	0.82	2%	0%
		220	11.3*	0.89	2%	0%
		460	32.5*	1.19	8%	0%
		1000	51.2*	0.88	23%*	6%
		4600	59.4*	3.32*	49%*	73%*
NTP (2006a)	153	0 µg/kg	1.00	1.00	0	0
		10	0.83	0.76	0%	0%
		100	1.12	0.97	0%	0%
		300	1.36	1.02	0%	0%
		1000	1.20	1.18	4%	0%
		3000	0.87	1.11	2%	0%

Study	Congener	Dose	EROD 53 wks fold [^]	Cell Proliferation 31 wks (fold [^]) N=10	Liver hepatocyte Tumors, A; [C]	Liver Carcinomas (cholangio)
NTP (2006e)	126+118	0 ng/ug	1.00	1.00	4%	0%
		10/ 62	24.4*	1.23	2%	0%
		30/ 187	38.3*	1.49	0%	0%
		100 622	47.4*	1.82	8%	2%
		300/ 1826	29.8*	18.1*	32%* [2%]	2%
		500/ 3110	missing	18.7*	8%	2%
NTP (2006d)	126 +153	0 ng/ug [G1]	1.0	1.0	0	0
		10/10 [G2]	-	-	0%	0%
		100/100 [G3]	-	-	6%	2%
		300/100 [G4]	44.3*	1.15	4% [†]	14% [†]
		300/300 [G5]	59.1*	0.78	10%* [†]	17%* [†]
		300/3000 [G6]**	19.1	3.52	41% [†]	49% [†]
		1000/1000 [G7]	- (~60;)	-	53%* [4%]	59%*
NTP (2006b)	TCDD	0 ng/kg	1.0	1.0	0%	0%
		3	18.9*	2.6*	0%	0%
		10	42.4*	2.92*	0%	0%
		22	51.3*	2.42*	0%	2%
		46	53.6*	4.07*	2%	8%*
		100	61.9*	11.8*	25%*	47%*

*Statistically significant changes are highlighted in yellow; [†] Statistically significant trend; A, adenoma; C, carcinoma.

** Data for tumors for groups 4 and 6 were included in Table 24 of NTP report – they looked for trends for the groups (but not difference to controls for groups 4 and 6)

They chose two DL-PCBs that account for the majority of Dioxin 'TEQ' in Aroclor 1254, PCB118 and PCB126, and one PCB congener, PCB153, that is the most prevalent of all congeners in Aroclors 1254 and 1260 but lacks any significant 'dioxin-like' activity. They also included TCDD itself, as well as two chlorinated diobenzofurans with significant 'dioxin-like' activity, and some studies with a mixture of two congeners. The results of these various studies are summarized in Table A3-9 (excluding the dibenzofuran studies).

The results can be summarized succinctly as follows: Liver (and benign lung) tumors were induced at the highest, and in some cases highest and second highest doses, with all PCBs and dioxins with AhR activity. PCB153, which lacks any appreciable AhR activity, did not induce liver or lung tumors in males or females at any dose. Tumor development (lung or liver, benign or malignant) required maximal stimulation of the AhR for most of the lifetime of the animals.

Since the purpose of the NTP (2006a, b, c, d, e, 2010) studies was to investigate the potential molecular mechanisms by which dioxins and dioxin-like compounds cause cancer, the investigators used extraordinarily high doses, relative to what people are exposed to in the environment. These studies found that TCDD, PCB126 and PCB118 caused a significant increase in liver tumors in female rats in a dose-related fashion.

Figure A3-9 shows that all three 'dioxin-like' compounds caused more than 50% of the animals to develop liver tumors at the highest doses, but that at doses that were 10-fold or more lower than the highest dose, no tumors appeared in any of the animals. These results demonstrate that there is a very steep 'dose-response', and a very clear 'threshold' (dose below which there is no response). Note that the 'dose' is plotted on a log scale, such that, at the 50% response level, TCDD is about 50 times more potent than PCB126 ($\sim 0.5/0.01$), and about 30,000 times ($\sim 300/0.01$) more potent than PCB118, based on '*in vivo*' liver tumor responses in female rats. This graph also illustrates well the concept of 'thresholds' – doses below which no effect is seen. Because dioxins and DL-PCBs tend to concentrate in the liver and other fatty tissues in the body, the plot shown here is the concentration of the toxic substance in the liver, rather than the daily dose that the animals received, but the relationship between administered dose and concentration in the liver is nearly perfectly linear over a wide range of doses, so the concept is the same.

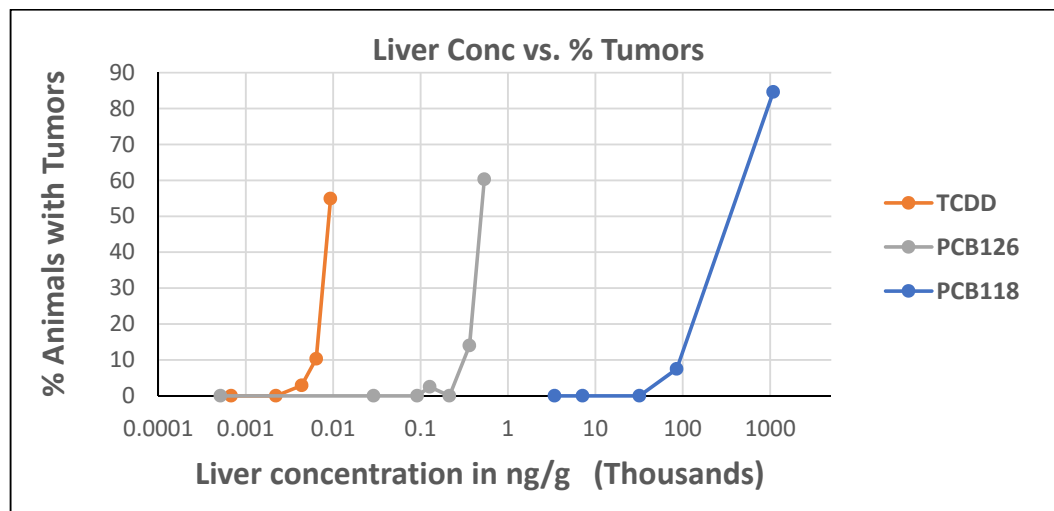


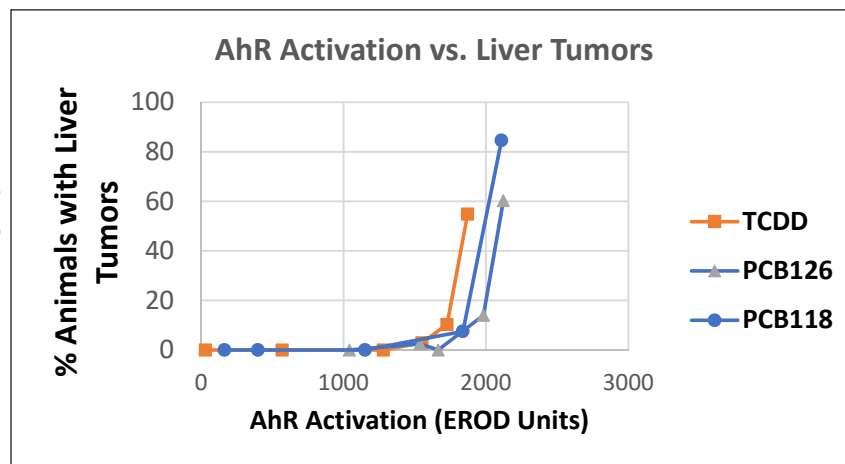
Figure A3-9. Dose response relationship between dioxin and dioxin-like PCBs concentration in the liver and liver tumor development in female rats at 105 weeks

The 'dose-response relationship' for activation of the AhR is very steep, and follows a 'threshold', rather than linear, relationship at low exposure levels; thus, the EPA's choice in 1996 to use a linear response to extrapolate the high dose animal studies to humans exposed to doses thousands of times lower is not supported by today's science

Since activation of the AhR by these 3 dioxin-like compounds is required for PCB mixtures to cause liver tumors, then one would expect that the number of tumors formed in each of these *in vivo* rat bioassays would correlate strongly with the level of activation of the AhR at the different doses used. Fortunately, the investigators at the NTP measured the extent of AhR activation in the livers of these animals at several different time points at each of the doses used. To measure 'AhR activation', they used an enzyme assay that measures the extent of induction of CYP1A1, using a chemical known as ethoxyresorufin. This molecule is specifically broken down (oxidized) by CYP1A1, and thus the enzymatic activity is called 'ethoxyresorufin-O-deethylase' activity, or simply EROD. EROD activity is a remarkably sensitive and robust measure of AhR activation in virtually any species of animal that has an AhR. Figure A3-10 shows a plot of the EROD activity (measured at 53 weeks of treatment) and the number of animals with liver tumors in each group (after up to

2 years of exposure). Note that the x-axis (horizontal axis) of the plot is NOT a log scale. This is a remarkable demonstration of the strong relationship between AhR activation (EROD activity) and the formation of liver tumors from all three dioxin-like compounds (TCDD, PCB126 and PCB118). Although these compounds differ by tens of thousands of times in their potency in activating the AhR, the range of activation necessary to cause liver tumors is less than 2-fold between the compounds, which falls well within experimental measurement error. This demonstrates that activation of the AhR by dioxin and dioxin-like compounds (e.g., PCB126 and PCB118) precedes tumor development, and thus appears necessary to cause liver tumors in rats; i.e., tumors appear only after significant and prolonged activation of the AhR. Thus, doses of DL-PCBs below the level necessary for maximal, prolonged activation of the AhR will not cause an increase in liver tumors, and thus there would be no increase in cancer risk. A second important observation from this graph is that, just as with tumor response and liver concentration, there is a very steep dose response curve, such that it requires a high level of activation of the AhR (EROD activity; greater than 1,000 units of activity, when the 'baseline' level is around 30-50 units) for tumors to develop, and follows the same 'threshold' type response as for tumor formation. In other words, a small amount of activation (e.g., less than 10 times the background) was not sufficient to cause tumors. This is a very important point when extrapolating tumor responses seen in rats at high doses, to try to estimate risk to humans at doses that are tens of thousands to hundreds of thousands of times lower than the dose the rats received in these and the other animal studies the EPA relied on for their quantitative risk assessment of PCBs in 1996.

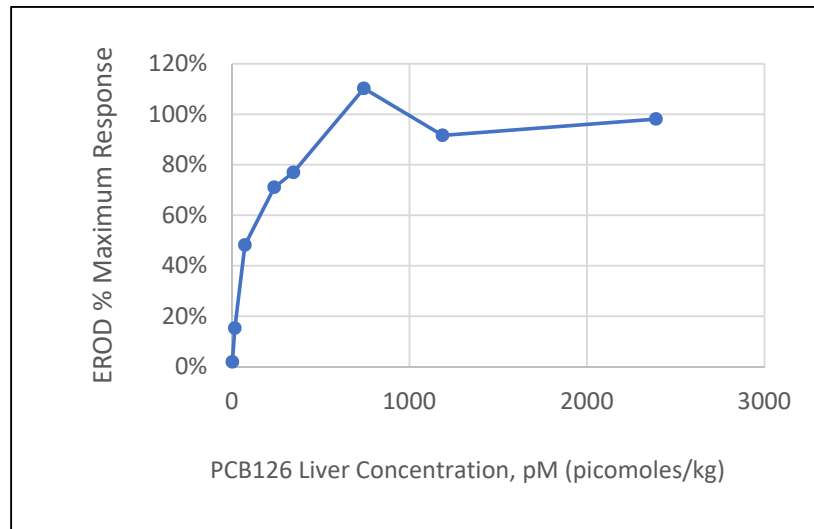
Figure A3-10.
Relationship
between liver tumor
formation and EROD
activity for three
different dioxin-like
compounds



These studies, when combined with the results of Mayes *et al.* (1998) and Brown *et al.* (2007) are remarkably informative, because they analyzed tissue concentrations of the PCB congeners in blood, liver, lung and fat, and also measured the extent of PCB congener induction of various cytochromes P450, including enzyme activity markers for CYP1A1 (EROD), 1A2 (Aniline Hydroxylase), and 2B + 3A (PROD) enzymes, which are regulated (fully or in part) by the ligand-activated transcription factors AhR, PXR and CAR. Most important is the EROD activity (CYP1A1-dependent), which is the 'gold standard' for measuring biologically relevant induction of CYP1A1, and is mediated exclusively by the AhR. Figure A3-11 represents data from the NTP study that examined EROD Activity (an enzymatic activity assay of EROD induction via AhR activation) in rat liver 53 weeks after daily treatment of rats with PCB126 at 8 different doses (0, 10, 30, 100, 175, 300, 550 and 1,000 ng/kg/d). The study also measured PCB126 concentrations in the 'target tissue' (liver, in this case,

but lung as well) as well as in blood. Figure A3-11 shows the dose-response curve for EROD induction by PCB126 vs. liver concentration.

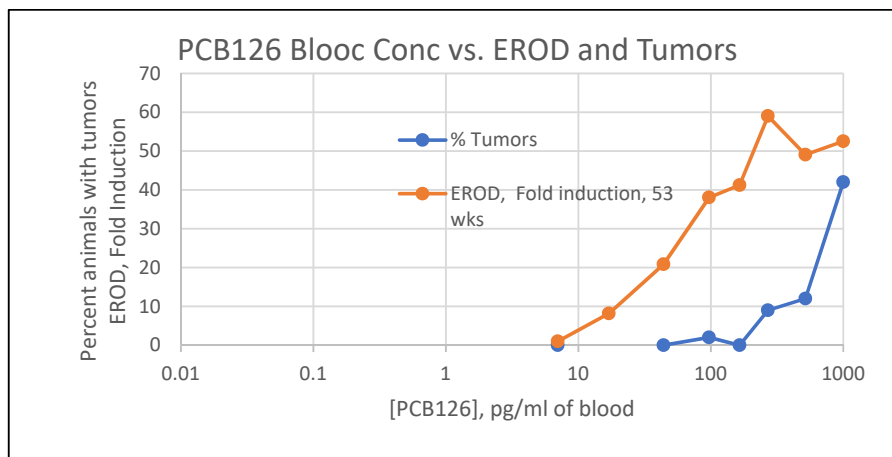
Figure A3-11. NTP PCB126 bioassay for EROD Activity, % maximum vs. liver PCB126 concentration after 52 weeks of dosing



It is evident that the lowest dose, 10 ng/kg/d, induced EROD activity to 16% of the maximum value, and the corresponding liver concentration was 16 pM. Extrapolation from the graph yields an *in vivo* BMD20 concentration of approximately 20 pM PCB126 in rat liver.

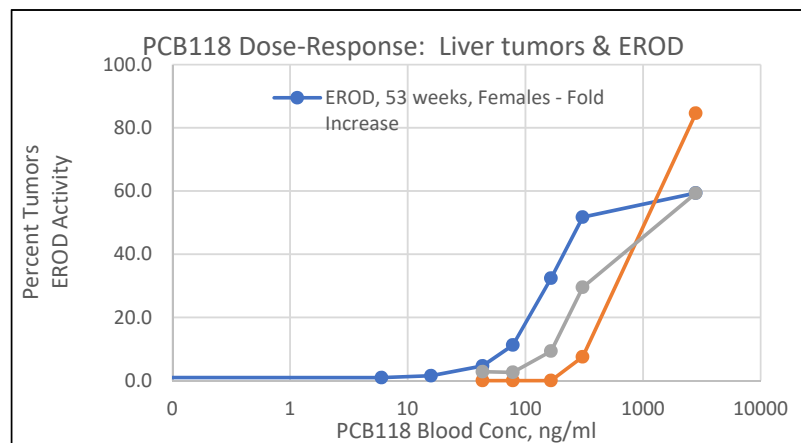
Since these studies measured EROD activity, liver concentrations, and tumor incidence, one can assess the dose-response relationship between AhR activation (CYP1A2-mediated EROD activity) and tumor development, and from that, determine both target tissue and blood concentrations necessary for both AhR activation and ultimate tumor formation. For example, Figure A3-10 illustrates the relationship between blood PCB126 concentration and both the induction of EROD (measured at 53 weeks of treatment) and the number of animals with liver tumors in each group (measured at the end of 2 years). It is evident that EROD induction (activation of the AhR) by PCB126 begins to become significantly different from baseline at a blood concentration of about 10 pg/ml, and that maximal induction (about 50-fold increase) is seen at a blood concentration of about 300 pg/ml. Another important point from this comparison is that tumors are not evident in animals until EROD activity has exceeded at least a 40-fold increase, near its maximal induction of 50-60 fold. In other words, not only is EROD activation necessary, but there must be complete, maximal and sustained activation before tumors are induced.

Figure A3-12.
PCB126 blood
concentration vs.
liver EROD
activity and
tumor formation
(data from NTP,
2006c)



Remarkably similar findings were observed in the study on PCB118 (Figure A3-13), except that blood concentrations required to initiate AhR induction were about 100 ng/ml (100,000 pg/ml), which is 10,000 times greater than PCB126, reflective of the relatively weak affinity of PCB118 for the rat AhR receptor (Larsson *et al.*, 2015). It is possible from these data to estimate PCB126 and PCB118 blood concentrations that are associated with a BMD20 for maximal induction of CYP1A1/EROD. Interpolating from the EROD fold-induction data in Figure A3-12 above, it is evident **that, in rats, a blood concentration of PCB126 of approximately 20 pg/ml (ppt) is associated with a ~10-fold induction of EROD, which is 20% of the maximal (~50X) induction.**

Figure A3-13.
PCB118 blood
concentration
versus EROD activity
and liver tumor
formation in female
Sprague-Dawley
rats

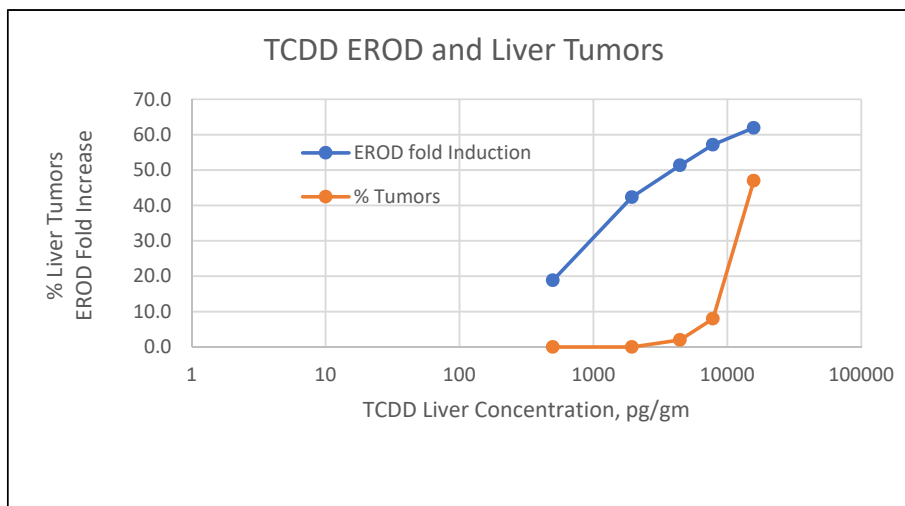


A similar calculation for PCB118 can be made from the data in Figure A3-13 above. Here, **a PCB118 blood concentration of ~80 ng/ml is associated with a 12-fold increase of EROD, which is 20% of the maximal (~60X) induction.**

Examination of the study on TCDD, which is essentially a 'pure' AhR agonist, yields yet again a very similar pattern in relationship between AhR activation (fold increase in EROD) and liver tumor formation: an increase

in EROD of greater than 50-fold is necessary for liver tumor development, and maximal induction is approximately 60-fold from baseline EROD activity (Figure A3-14).

Figure A3-14.
Liver tumor
formation and
EROD induction
vs.
concentration
of TCDD in the
liver



Alternative (non-AhR-mediated) modes of action for PCB carcinogenesis

The Budinsky *et al.* (2014) evaluation of dioxin carcinogenesis provides highly relevant conclusions that are pertinent to risk assessment of PCBs, since there is now overwhelming scientific evidence that the carcinogenic effects of PCBs in laboratory animals are mediated via the AhR as the key initiating molecular event. However, when using an AOP/MOA approach to risk assessment, IPSC and EPA suggest that alternative MOAs for the endpoint of interest be considered and ‘ruled out.’ One potential alternative MOA for PCB carcinogenesis is mutagenesis. Although there is some evidence that lower chlorinated PCBs may be mutagenic under some circumstances (see Section A of this Appendix [Appendix 3] starting on p. 182), the preponderance of the evidence for PCBs that bioaccumulate in the environment and are found in fish is that genotoxicity/ mutagenicity do not constitute a likely MOA for observed carcinogenesis in laboratory animals exposed to higher chlorinated Aroclors (e.g., 1242, 1254, and 1260) or the congener mixture that is present in fish following environmental bioaccumulation. This conclusion is supported by several *in vivo* studies that have looked for, but found no evidence of, genotoxicity in humans following relatively high levels of dietary exposure to PCBs (Ravoori *et al.*, 2008; Rignell-Hydbom *et al.*, 2005). Further, if mutagenicity was a significant contributor to the rodent carcinogenic responses, one would expect to see significant increase in liver tumors in the Mayes *et al.* (1998) study of Aroclor 1016, and in the NTP (2006a) carcinogenesis assay of PCB153, both of which were negative. Thus, the preponderance of the evidence on carcinogenic mode of action for PCBs is via activation of the AhR and the subsequent well-characterized ‘downstream’ events that ultimately lead to tumor development in rat liver.

This was also addressed thoroughly in the Budinsky *et al.* (2014) workshop on dioxin carcinogenesis, where mutagenesis was evaluated as a potential MOA for dioxin carcinogenesis. The workshop conclusions on this point state the following:

“Alternate MOA(s) must be examined as part of the MOA and human relevance framework approach (Boobis et al., 2006, 2009; Cohen et al., 2003, 2004; Holsapple et al., 2006; Julien et al., 2009; Meek, 2008; Meek et al., 2003; Seed et al., 2005; Sonich-Mullin et al., 2001; US EPA, 2005). The expert panel concluded that the MOA for liver carcinogenesis is not due to direct acting genotoxicity, but rather via sustained AHR activation and is most likely to be non-linear. The evidence for this has been discussed earlier. The expert panel reviewed the relevance of oxidative stress and ROS production as alternative MOAs (see discussion earlier under ModFs) since they can be linked to mutagenicity and genotoxicity. Potential mutagenicity of TCDD, although unlikely, was considered as an alternative MOA because of the potential to alter the low-dose extrapolation method and thus, risk assessment results. As discussed, no evidence for DNA adducts formed by catechol estrogens binding to DNA was found using a sensitive accelerator mass spectrometry method (Turteltaub et al., 1990). Endogenous DNA adducts (I compounds) were reduced following sub-chronic TCDD treatment (Randerath et al., 1990).

The panel agreed that the weight of evidence, including a number of mutagenicity and genotoxicity studies of dioxin-like chemicals, suggest that these chemicals are not mutagenic (Bock & Kohle, 2005, 2006; Cohen, 1998; Dragan & Schrenk, 2000; Knerr & Schrenk, 2006; Schwarz et al., 2000; Whysner & Williams, 1996). This conclusion is consistent with recent regulatory assessments of dioxins. A framework for the consideration of DNA adducts as part of a carcinogenic MOA has been developed (Himmelstein et al., 2009; Jarabek et al., 2009; Swenberg et al., 2008, 2011). Examination of potential KEs in the MOA that could be related to DNA damage induced by dioxin, the formation of DNA adducts or mutations within the context of this framework, could not confirm a DNA-reactive or mutagenic MOA for dioxin.”

Activation of PXR and/or CAR via non-DL-PCBs as a putative MOA for PCB carcinogenesis. Since PCBs contain non-dioxin like, as well as the AhR-activating DL-PCBs, one must consider whether non-DL-PCBs themselves might be carcinogenic via non-AhR pathway(s). The Pregnane-X-Receptor (PXR; aka SXR) and the the Constitutive Androstane Receptor (CAR), like the AhR, are ligand-activated nuclear transcription factors. When an activating ligand of PXR or CAR binds to the receptor, a series of downstream events occur as a result of changes in gene transcription, conceptually the same as the AhR. However, the genes that are regulated by PXR and CAR are different than those regulated by AhR.

Constitutive Androstane Receptor (CAR): It has been known for decades that Aroclor PCB mixtures, when given to rats at doses in excess of about 1 mg/kg/day, causes a change in the liver referred to as ‘microsomal enzyme induction’, also called ‘mixed-function oxidase’ (MFO) induction. These terms refer to the molecular processes by which many drugs and exogenous chemicals, including nearly all mixtures of PCBs, turn on a large set of genes that make proteins in the ‘endoplasmic reticulum’ of the liver. The magnitude of this effect at higher doses is substantial, resulting in an increase in the weight of the liver. Many of the proteins in the endoplasmic reticulum of the liver are involved in xenobiotic metabolism, with the purpose of detoxifying foreign substances that enter the body through ingestion. One important family of genes, known as the ‘Cytochromes P450’, or simply CYPs, makes CYP proteins that function to oxidize both endogenous and exogenous chemicals. Through this process of oxidation, fat-soluble chemicals are ultimately made water-

soluble so that they can be quickly eliminated from the body. It is the resistance to oxidation by CYPs and other oxidative enzymes that makes PCBs persistent in the environment (and it is also this same resistance to oxidation that made PCBs so useful for a variety of industrial purposes). In the early days following the discovery of MFOs/CYP enzymes, it was thought that there were only two different genes responsible for this enzyme activity, the so-called Cytochrome P450 and Cytochrome P448. The first (CYP450) was 'inducible' (could be turn on) by the widely used barbiturate, phenobarbital, whereas the second (CYP448) could be induced by a polyaromatic hydrocarbon called 3-methylcholanthrene (3MC). Thus, in the early days (pre-1970s) of drug metabolism studies drugs and chemicals that induced CYP450 were referred to as 'phenobarbital-like' and drugs and chemicals that induced CYP448 were referred to as '3MC-like.' When Aroclor mixtures were first examined for their effects on drug metabolizing enzymes, it was discovered that PCBs were 'mixed inducers', that is they induced BOTH CYP450 and CYP448. Over the ensuing decades researchers discovered that there are 88 different genes in the rat genome that code for CYP proteins (Nelson *et al.*, 2004), and about a dozen are normally expressed in adult rat liver (that code for CYP enzymes, not just 2). These enzymes fell into different classes and sub-classes, based on their DNA sequences. Thus, today CYP enzymes have multiple letters and numbers to specify the particular gene involved in making that protein. It is now recognized that the old 'CYP448' term actually represents several different P450 genes that are regulated by the Arylhydrocarbon Receptor (AhR), most important of which are CYP1A1, CYP1A2 and CYP1B1. The importance of the AhR in liver tumor development in the rat following exposures to PCB mixtures with DL-PCBs has already been discussed.

However, other PCBs, most notably the non-DL-PCB, PCB153 can interact with and activate rat CAR, resulting in an increase in the expression of different P450s, with a pattern of induction very similar to that seen with Phenobarbital. Since it has been known for decades that phenobarbital acts as a liver tumor promoter in rats, there was great interest in understanding whether 'phenobarbital-like PCBs' (e.g., PCB153) might also act as liver tumor promoters. A great deal of research has been conducted to understand the exact molecular events involved in phenobarbital promotion of rat liver tumors. However, there were also numerous human epidemiology studies on chronic users of phenobarbital (used in the treatment of epilepsy and other neurological disorders) which repeatedly failed to find any evidence of increased cancer risk in chronic users of phenobarbital and similar barbiturates, calling into question whether CAR induction in humans was actually a risk factor for liver (or other) cancer. To understand the relevance of CAR activation in rats to human liver cancer, an international group of experts was assembled to assess the scientific evidence regarding the relevance of CAR activation in the rat by phenobarbital or other CAR agonists (such as 'phenobarbital-like PCBs'). Using the 'Mode of Action' analysis described above for the AhR receptor, the committee weighed all of the scientific evidence available at that time, and published a majority report that summarized the current state of knowledge about CAR activation and liver cancer risk in humans Elcombe *et al.* (2014). Using the prototype for understanding human risks from Nuclear Receptor Activation reported by Andersen *et al.* (2013) the expert committee provided the following 'flow chart' for the Mode of Action (MoA) of CAR activators in causing liver cancer in rodents:

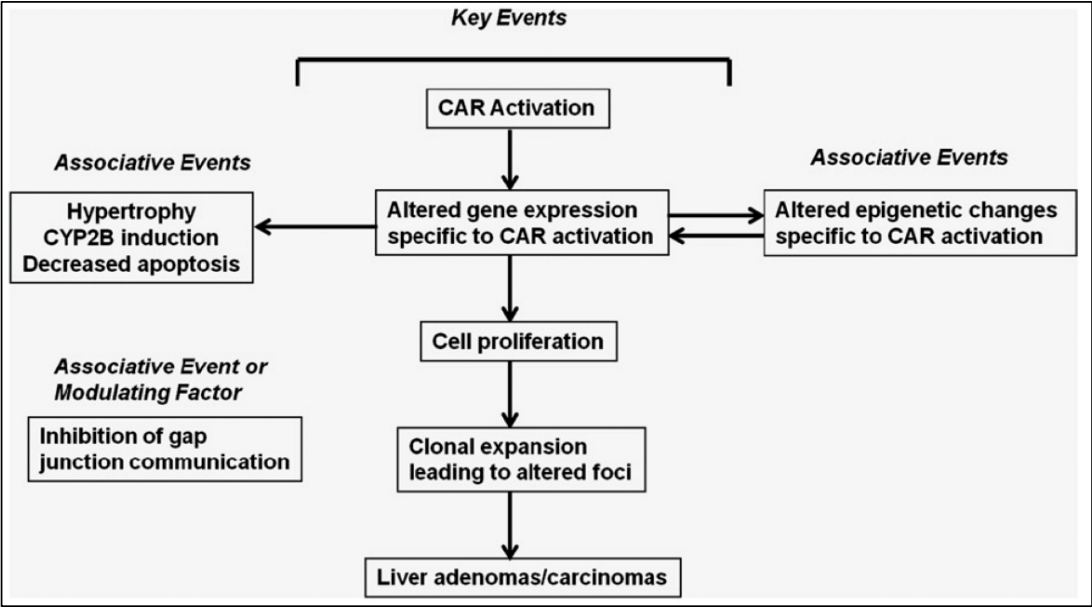


Figure A3-15. MoA for Phenobarbital-induced rodent liver tumor formation. Proposed key events, associative events and modulating factors for the mode of action (MOA) for PB-induced rodent liver tumor formation. The initial key event is CAR activation which results in altered gene expression, increased cell proliferation, clonal expansion? leading to altered foci and subsequently in the formation of liver tumors. Associative events which can serve as reliable biomarkers of key events include epigenetic changes, induction of CYP2B enzymes and liver hypertrophy and decreased apoptosis; whereas inhibition of gap junctional intercellular communication constitutes an associative event or modulating factor (from: Elcombe *et al.*, 2014)

The Committee then evaluated all of the existing literature to evaluate the relevance of this MoA for CAR activators to human cancer risk, and provided the following analysis, using the IPSC risk analysis process described by Andersen *et al.* (2013), but applied specifically to CAR activators:

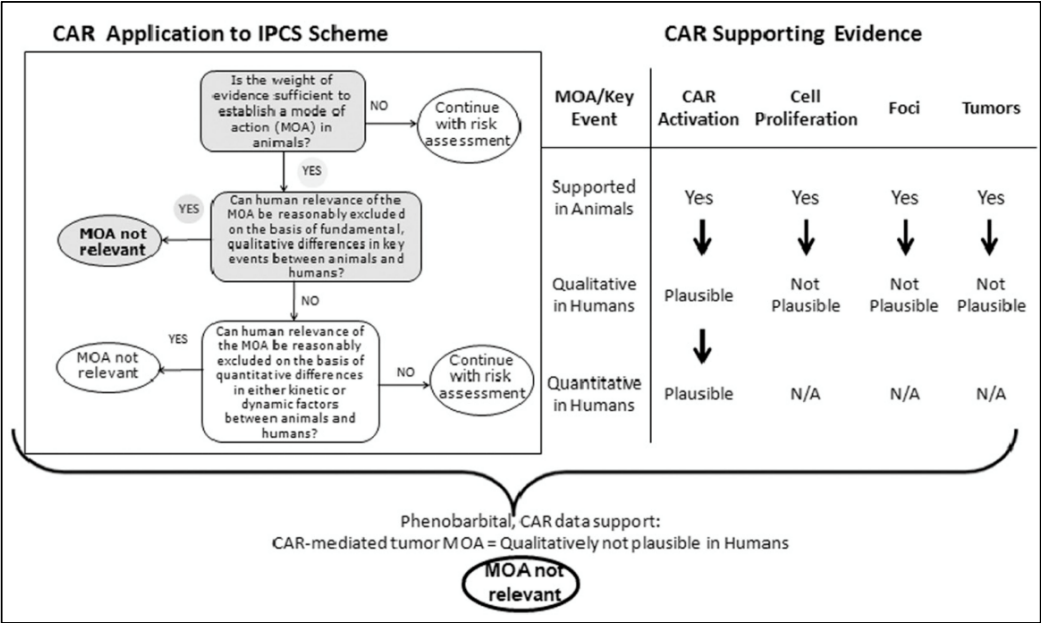


Figure A3-16 Human relevance framework analysis for Phenobarbital-induced rodent liver tumor formation (from: Elcombe *et al.*, 2014)

Based on this analysis the committee provided the following conclusions regarding the relevance of CAR activators, such as PCB153 and other ‘phenobarbital-like’ PCBs (Elcombe *et al.*, 2014):

In conclusion, from an evaluation of literature data a robust MOA based on CAR activation for PB-induced rodent liver tumor formation has been developed. The data on species differences was considered by the majority of the panel to be sufficient to determine that this MOA would be qualitatively not plausible for humans. Thus compounds that cause rat or mouse liver tumors through this CAR-mediated MOA, similar to PB, would not be expected to increase the risk of liver tumor development in humans.

Members of the panel and their affiliations are shown in Table A3-10:

Table A3-10. CAR/PXR case study panel members and affiliations (from: Elcombe *et al.*, 2014)

	Participant names	Affiliations
Co-Chairs	Cliff Elcombe, PhD	CXR Biosciences
	Douglas Wolf, DVM, PhD,	U.S. EPA
Rapporteurs	Jillian McEwan, PhD	CXR Biosciences
	Audrey Vardy, PhD	CXR Biosciences
Panel Members	Jason Bailey, PhD	Dow Agrosciences
	Remi Bars, PharmD, PhD	Bayer CropScience
	David Bell, PhD	European Chemicals Agency
	Russell Cattley, DVM, PhD	Auburn University
	Rory Conolly, ScD	U.S. EPA
	Kenny Crump, PhD	Louisiana Tech University
	Stephen Ferguson, PhD	CellzDirect/Life Technologies
	David Geter, PhD	Dow Chemical Company
	Amber Goetz, PhD	Syngenta Crop Protection Inc.
	Jay Goodman, PhD	Michigan State University
	Susan Hester, PhD	U.S. EPA
	Abigail Jacobs, PhD	U.S. FDA-CDER
	Brian Lake, DSc	Centre for Toxicology, University of Surrey
	Curtis Omiecinski, PhD	Molecular Toxicology and Carcinogenesis, Penn State University
	Richard Pepper, PhD	Syngenta Crop Protection, LLC
	Rita Schoeny, PhD	U.S. EPA
	Wen Xie, MD, PhD	Center for Pharmacogenetics, University of Pittsburgh

Supporting the conclusions of this panel that CAR activation is not a human-relevant MoA for liver tumors observed in rats following CAR activation, Brown *et al.* (2007) provided a remarkably detailed analysis of the chain of molecular events that unfold in male and female rats following long-term administration of Aroclors. As discussed above under the AhR MoA, Brown *et al.* (2007) used molecular analysis data obtained from the rats used in the Mayes *et al.* (1998) 2-year bioassay of Aroclors 1016, 1242, 1248, 1254 and 1260. Their analyses included multiple different measures of CYP induction, generation of reactive oxygen species (ROS; responsible for 'oxidative stress' in a tissue/organ), and multiple other measures to assess the chain of molecular events that ultimately lead to liver cancer in rats in a species and sex-related fashion (Figure A3-5 and Figure A3-6). That analysis, plus the CAR evaluation provided above, demonstrate that CAR activation by PCBs in humans has no relevance to human risk assessment of PCBs found in fish. A similar conclusion can be reached about nuclear receptor PXR activation, since Tabb *et al.* (2004) demonstrated that, although some PCBs activate rat PXR, they do not activate human PXR, and in fact may act as inhibitors of human PXR, at least at high doses.

Finally, Ayotte *et al.* (2005) did an extensive analysis of a fish-eating population living on The Lower North Shore region of the St. Lawrence River with an unusually high body burden of polychlorinated biphenyls (PCBs) and dioxin-like compounds (DLCs). They measured biomarkers indicative of liver enzyme induction and

investigated the relationship with organochlorine body burden in adult volunteers. The results of their analyses led to the following conclusion: *"In summary, we found no relation between biomarkers of OC [organochlorine compound] exposure and markers of hepatic enzyme induction in this highly exposed group of fish eaters from the Lower North Shore of the St. Lawrence River."* Given the very high levels of PCB exposure in this high fish-eating population, relative to consumers of Spokane River fish, the lack of any notable change in biomarkers of drug metabolizing enzymes provides further proof that activation of CAR, PXR and/or AhR does not occur at the doses of PCBs provided through consumption of fish from the Spokane River.

Thus, there is now overwhelming evidence that the only relevant Mode of Action for PCBs as potential human carcinogens is via activation of the AhR, and thus the only relevant PCBs for human risk assessment purposes are the DL-PCBs.




D. Species differences in AhR responsiveness to dioxins and DL-PCBs:

It is important to realize that ALL REPs/TEFs/TEQs discussed in the literature, established by the WHO, and used by the US EPA for risk assessment purposes, are based on studies in laboratory rats. But it has been recognized for decades that there are important species differences in susceptibility to dioxins and dioxin-like molecules that activate the AhR. Soon after the WHO Expert Review panel established the 2005 TEFs Table A3-11, (van den Berg *et al.*, 2006), discussed the many deficiencies in the WHO TEFs, especially noting the importance of species differences:

“Literature data also indicate that the PCB126 REP [Relative Effective Potency] for enzyme induction in human cell systems, including primary hepatocytes, breast cancer cell lines and primary lymphocytes, may be one or two orders of magnitude lower

(Zeiger et al., 2001; van Duursen et al., 2003). In addition, the apparent binding affinity of

Table A3-11. Previous WHO TEFs for dioxins, dibenzofurans and DL-PCBs (from: WHO, 2019)

The International Programme on Chemical Safety (IPCS)		 WHO		 UNEP
Compound	WHO 1998 TEF	WHO 2005 TEF*		
<i>chlorinated dibenzo-p-dioxins</i>				
2,3,7,8-TCDD	1	1		
1,2,3,7,8-PeCDD	1	1		
1,2,3,4,7,8-HxCDD	0.1	0.1		
1,2,3,6,7,8-HxCDD	0.1	0.1		
1,2,3,7,8,9-HxCDD	0.1	0.1		
1,2,3,4,6,7,8-HpCDD	0.01	0.01		
OCDD	0.0001	0.0003		
<i>chlorinated dibenzofurans</i>				
2,3,7,8-TCDF	0.1	0.1		
1,2,3,7,8-PeCDF	0.05	0.03		
2,3,4,7,8-PeCDF	0.5	0.3		
1,2,3,4,7,8-HxCDF	0.1	0.1		
1,2,3,6,7,8-HxCDF	0.1	0.1		
1,2,3,7,8,9-HxCDF	0.1	0.1		
2,3,4,6,7,8-HxCDF	0.1	0.1		
1,2,3,4,6,7,8-HpCDF	0.01	0.01		
1,2,3,4,7,8,9-HpCDF	0.01	0.01		
OCDF	0.0001	0.0003		
<i>non-ortho substituted PCBs</i>				
PCB 77	0.0001	0.0001		
PCB 81	0.0001	0.0003		
PCB 126	0.1	0.1		
PCB 169	0.01	0.03		
<i>mono-ortho substituted PCBs</i>				
105	0.0001	0.00003		
114	0.0005	0.00003		
118	0.0001	0.00003		
123	0.0001	0.00003		
156	0.0005	0.00003		
157	0.0005	0.00003		
167	0.00001	0.00003		
189	0.0001	0.00003		

* Numbers in bold indicate a change in TEF value

Reference - Van den Berg *et al.*:

The 2005 World Health Organization Re-evaluation of Human and Mammalian Toxic Equivalency Factors for Dioxins and Dioxin-like Compounds

2,3,7,8-TCDD to the human AhR is generally 1/10th that of the AhR of the more sensitive rodent species

But significant variation among individual humans occurs (Roberts et al., 1990; Ema et al., 1994; Poland et al., 1994; Harper et al., 2002; Ramadoss and Perdew, 2004). It has been suggested that on average humans are among the more dioxin-resistant species, but the human data set is too limited to be conclusive (Harper et al., 2002; Okey et al., 2005). A study with AhR-humanized mice may indicate lower responsiveness towards toxic effects of 2,3,7,8-TCDD (Moriguchi et al., 2003). Taken together this information warrants more research into REP values in human systems to establish if the present TEFs based on rodent studies are indeed also valid for humans” [emphasis added]

In 2015, Larsson et al. (2015) published a ground-breaking paper that included a detailed assessment of Relative Effective Potency (REP) values for various dioxins, dibenzofurans, and dioxin-like PCB congeners that are used to establish the TEFs, using direct comparisons of human and rat cells, in a variety of cell types (Table A3-12).

As noted previously, it has long been known that human AhR has different affinities for some ligands than rat AhR (Denison et al., 2011; van den Berg et al., 2006; van Duursen et al., 2017). van Duursen et al. (2017) recently commented on the outdated use of rodent TEFs in human risk assessments for PCBs: “These rodent-derived TEFs are applied world-wide for human risk assessment purposes. This in spite of the fact that upon AHR activation, a wide variety of toxic and biological effects can occur that display clear differences in rodent and human responses.” The 2015 study by Larsson et al. was the first to thoroughly demonstrate, quantitatively, the dramatic difference in response to DL-PCBs between rats and humans. This is important because the large majority of risk assessments for the various adverse outcomes from PCBs are based on dose-response modeling of rat responses, with an assumption that the human AhR has similar Relative Effective Potency (REP) to that of rat.

After thorough testing of the different DL-PCBs in 17 different assays of rat and human cells, this group (which included as second author Martin van den Berg, the leading authority in the world

Table A3-12. Consensus toxicity factors for compounds with WHO TEFs (from: Larsson et al., 2015)

compound ^a	CTF		WHO-TEF
	rat	human	
Chlorinated Dibenzo- <i>p</i> -dioxins			
2378-TCDD ^b	1	1	1
12378-PeCDD ^b	0.5	1	1
123478-HxCDD ^c	0.2	0.03	0.1
123678-HxCDD ^b	0.06	0.06	0.1
123789-HxCDD ^c	0.3	0.002	0.1
1234678-HpCDD ^b	0.04	0.2	0.01
OCDD ^c	— ^e	0.005	0.0003
Chlorinated Dibenzofurans			
2378-TCDF ^b	0.2	0.1	0.1
12378-PeCDF ^c	0.2	0.6 ^{ax}	0.03
23478-PeCDF ^b	0.2	1	0.3
123478-HxCDF ^b	0.09	1	0.1
234678-HxCDF ^b	0.07	0.06	0.1
123678-HxCDF ^c	0.07	0.04 ^{ax}	0.1
123789-HxCDF ^c	0.3	0.02	0.1
1234678-HpCDF ^b	0.01	0.01	0.01
1234789-HpCDF ^b	0.05	0.3	0.01
OCDF ^c	0.007 ^{ax}	0.2 ^{ax}	0.0003
Non- <i>ortho</i> -substituted PCBs			
PCB77 ^b	0.0004	— ^d	0.0001
PCB81 ^c	0.0002	— ^d	0.0003
PCB126 ^b	0.09	0.003	0.1
PCB169 ^b	0.002	— ^d	0.03
Mono- <i>ortho</i> -substituted PCBs			
PCB74 ^b	0.000004	— ^d	—
PCB105 ^b	0.00001	— ^d	0.00003
PCB114 ^c	0.00006	— ^d	0.00003
PCB118 ^b	0.000009	— ^d	0.00003
PCB123 ^c	0.000009	— ^d	0.00003
PCB156 ^b	0.00008	— ^d	0.00003
PCB157 ^c	0.00003	— ^d	0.00003
PCB167 ^b	0.000007	— ^d	0.00003
PCB189 ^b	0.000007	— ^d	0.00003

on the TEF approach), arrived at 'Consensus TEFs' for both human and rat, and compared them to the 2005 WHO TEF values (Table A3-12).

1. Calculation of a 'species correction factor' to adjust for the large difference in DL-PCB affinity toward AhR between rat and human

Although the TEF values reported by Larsson *et al.* (2015) appear to suggest that the difference in PCB126 ligand affinity between rat and human is 0.003, that is not correct. The 0.003 value is the relative difference in PCB126 affinity compared to TCDD in the human receptor. But, as discussed previously, there is a substantial difference in potency of TCDD between rat and human with the human AhR response about 26-fold less than that seen toward the rat for the same concentration of TCDD. Thus, in order to compare a 'rat response' with a human response to a DL-PCB, it is necessary to compare the rat response for that ligand (e.g., PCB126 or PCB118) with the human response to those same DL-PCBs.

Table A3-13 (from: Larsson *et al.*, 2015) reveals some remarkable differences between human and rat AhR response to the DL-PCBs that dramatically changes human risk assessments for PCBs from those done by EPA in 1996, and others that are based solely on rat TEFs.

Table A3-13. Responsiveness of rat, mouse, guinea pig and human tissues to various dioxins, dibenzofurans and dioxin-like PCBs (from: Larsson *et al.*, 2015)

compound ^b	rat liver epithelial cells		rat lung epithelial cells		rat liver		rat liver	mouse liver	guinea pig liver	human liver		human keratinocytes	
	Cyp1a1 mRNA ^e	Cyp1b1 mRNA ^e	Cyp1a1 mRNA ^f	Cyp1b1 mRNA ^f	EROD primary hepatocytes ^g	EROD H4IIE hepatoma cells ^g	Luc. ^h	Luc. ^h	Luc. ^h	EROD Primary hepatocytes ^g	Luc. HepG2 AZ-AhR cells	CYP1A1 mRNA	AhRR mRNA
Chlorinated Dibenzo- <i>p</i> -dioxins													
2378-TCDD	0.020	0.0019	0.0062	0.0032	0.0042	0.0038	0.0056	0.011	0.0015	0.11	0.19	0.12	0.10
12378-PeCDD	0.032	0.0050	0.012	0.0054	0.013	0.0060	0.012	0.0091	0.0018	0.058	0.069	0.064	0.039
123678-HxCDD	1.3	0.10	0.11	0.023	0.052	0.039	0.052	0.030	0.014	1.5	1.3	4.7	— ⁱ
1234678-HpCDD	0.17	0.0079	0.11	0.0076	0.14	0.080	0.20	0.095	0.019	0.70	0.59	0.42	0.15
Chlorinated Dibenzofurans													
2378-TCDF	0.018	0.0010	0.0083	0.0036	0.025	0.083	0.10	0.013	0.0056	0.89	1.8	0.73	1.5
23478-PeCDF	0.030	0.0059	0.13	0.058	0.045	0.0059	0.036	0.0096	0.0012	0.050	0.48	0.055	0.15
123478-HxCDF	0.11	0.0069	0.025	0.0056	0.13	0.042	0.075	0.025	0.0050	0.14	0.12	0.057	0.075
234678-HxCDF	0.091	0.00020	0.040	0.0047	0.14	0.073	0.096	0.018	0.0059	1.8	2.3	0.69	1.4
1234678-HpCDF	0.52	0.027	0.47	0.10	0.58	0.37	0.38	0.43	0.020	11	8.8	2.1	2.0
1234789-HpCDF	0.15	0.010	0.10	0.033	0.42	0.041	0.15	0.066	0.013	0.43	0.39	0.22	0.35
Non-ortho PCBs													
PCB77	2.4	0.13	1.1	0.28	9.28	67	40	6.0	0.71	— ^c	— ^c	— ^c	— ^c
PCB126	0.18	0.0030	0.027	0.0081	0.073	0.041	0.076	0.24	0.0081	26	58	50	— ^c
PCB169	2.6	0.058	1.38	0.50	5.8	4.7	2.6	21	0.11	— ^c	— ^c	— ^c	— ^c
Mono-ortho PCBs													
PCB74	14	0.17	1.4	0.26	— ^c	— ^c	4.2	4.5	1.1	— ^c	— ^c	— ^c	— ^c
PCB105	1.8	0.045	0.62	0.089	0.80	— ^c	1.3	6.1	0.012	— ^c	— ^c	— ^d	— ^d
PCB118	— ^c	— ^c	0.56	0.15	1.3	— ^c	1.6	2.9	0.13	— ^c	— ^c	— ^d	— ^d
PCB156	0.20	— ^c	0.046	0.0071	0.039	0.081	0.048	0.28	0.011	— ^c	— ^c	— ^d	— ^d
PCB167	— ^c	0.46	5.0	0.45	— ^c	— ^c	1.5	7.8	0.12	— ^c	— ^c	— ^d	— ^d
PCB189	5.5	0.072	0.87	0.085	— ^c	— ^c	— ^c	— ^c	0.12	— ^c	— ^c	— ^d	— ^d
Di-ortho PCB													
PCB153	— ^c	— ^c	— ^c	12	— ^c	— ^c	— ^c	— ^c	— ^c	— ^c	— ^c	— ^d	— ^d

The two major differences of relevance to this report are: 1) only one DL-PCB, PCB126, was capable of activating the human AhR, even though the other DL-PCBs activated the rat AhR with relative potencies very similar to previous WHO determinations. All other DL-PCBs were “too weak to calculate a BMR_{20TCDD} ” (footnote c; Table 1 of Larsson *et al.*, 2015); and 2) while PCB126 was able to activate human AhR, it did so with a relative potency approximately 666 times less than rat (Table 1, footnote c and Table 4 of Larsson *et al.*, 2015).²⁴

Larsson *et al.* (2015) used 3 different rat liver assays to determine a BMD20 for AhR activation by PCB126. The values were 0.073, 0.041 and 0.076 nM (Table A3-13). The average for rat liver response is thus 0.063 nM. They also used 2 different assays of human liver cells, with values of 26 and 58 nM, with an average of 42 nM. Thus, using the average value of different liver cell assays for each species, the difference in species sensitivity to PCB126 activation of AhR in the liver is $42/0.063 = 666$. For PCB126, comparing the human AhR response to the response in rat to TCDD, one arrives at a species difference TEF of: Human EROD response = 42nM / rat TCDD response of 0.0042 nM = 10,000. Thus, the Larsson *et al.* (2015) data suggests that, to compare a dose of PCB126 in humans to the response of rats to TCDD, the ‘correction’ value would be ~10,000; in other words, the human AhR is 10,000 times less responsive to PCB126 when compared to the rat response to TCDD. The relative potency of TCDD in activating the human, versus the rat, AhR in isolated rat hepatocytes was $0.11 [\text{human}] / 0.0042[\text{rat}] = 26$ -fold difference. In other words, to correct for cross species extrapolation, it takes 26 times more TCDD for the human AhR receptor to cause the same level of activation of the rat AhR.

It is important to recognize that the values TEF values for DL-PCBs reported in 2015 by Larsson *et al.* (2015) are very consistent with previous studies that have noted large differences in AhR activation between human and rat liver. For example, Carlson *et al.* (2009) conducted similar experiments with both rat and human hepatocytes, using TCDD and PCB126. They reported a ‘Relative Effective Potency (REP) value for PCB126, compared to TCDD, in humans of 0.0022 (95% CI 0.001, 0.005), and for the rat AhR 0.057 (95% CI 0.03, 0.1). These values are very similar to what was reported by Larsson *et al.* (2015), validating the use of a TEF of 0.003 for PCB126 toward the human AhR. Shi *et al.* (2019) also determined a human AhR REP value for PCB126 of 0.002, further validating the findings of Larsson *et al.* (2015). A recent publication by Brennan *et al.* (2015) reported similar findings using a CALUX assay for both rat and human AhR, in that the human receptor response to TCDD was about 10-fold lower than the rat, and PCB126 was highly active toward the rat receptor, but had barely measurable activity toward the human receptor at 100-fold higher concentration. Wimmerova *et al.* (2016) determined REPs for dioxin-like compounds (DLCs) using expression of cytochrome P450 (CYP) 1A1 and 1B1 mRNA in human peripheral blood mononuclear cells representing two different pathways. They used a sex and age adjusted regression-based approach comparing the strength of association between each DLC and the cytochrome P450 (CYP) 1A1 and 1B1 mRNA expression in 320 adults residing in an organochlorine-polluted area of eastern Slovakia. Remarkably, this study found strong human ‘*in vivo*’ correlations between the Relative Effective Potencies (REPs, or TEFs) calculated using human and rat

²⁴ Larsson, *et al.* (2015) used 3 different rat liver assays to determine a BMD20 for AhR activation by PCB126. The values were 0.073, 0.041 and 0.076 nM. The average for rat liver response is thus 0.063 nM. They also used 2 different assays of human liver cells, with values of 26 and 58 nM, with an average of 42 nM. Thus, using the average value of different liver cell assays for each species, the difference in species sensitivity to PCB126 activation of AhR in the liver is $42/0.063 = 666$, yielding a TEF value of 0.0015.

tissues *in vitro* (Larsson *et al.*, 2015), and the values obtained from their *in vivo* correlative studies. In short, the 0.003 TEF for PCB126 was confirmed '*in vivo*', and the lack of any significant AhR activation for the other 10 'dioxin-like' PCBs, as demonstrated *in vitro* by Larsson *et al.* (2015), was also confirmed.

Although it appears from the way REPs in Table A3-12 are presented that human and rat AhR are equally responsive to TCDD (both values appear as 1), that is because the 'relative potency values' are relative to the species-specific response to TCDD in the assays (thus, all 'dioxin-like' chemicals are compared to the value obtained for TCDD in that species). The data in Table A3-13 demonstrate that the human liver AhR response to TCDD is approximately 26-fold less sensitive to AhR activation by TCDD, consistent with reports from decades earlier suggesting that humans were 10-100-times less sensitive to dioxins, compared to rats. For example, Budinsky *et al.* (2010) conducted a series of studies with human (N= 5) and rat liver cells in primary culture and compared the relative potency of TCDD and two chlorinated dibenzofurans (PeCDF and TCDF) between rats and humans. They reported average 'EC50' for activation of human AhR (measured by several different means, including CYP1A1 mRNA and EROD activity) for TCDD, 4- PeCDF, and TCDF of 0.37, 0.67, and 2.02 nM, respectively and for rats, the values were 0.012, 0.43, and 0.09nM, respectively (Figure A3-17). Thus, the Human:Rat ratio of EC50s for AhR induction by TCDD was $0.37/0.012 = 30$. This is essentially the same value for Human:Rat potency of TCDD toward the AhR as found by Larsson *et al.* (2015), who reported 26 fold difference between human and rat AhR.

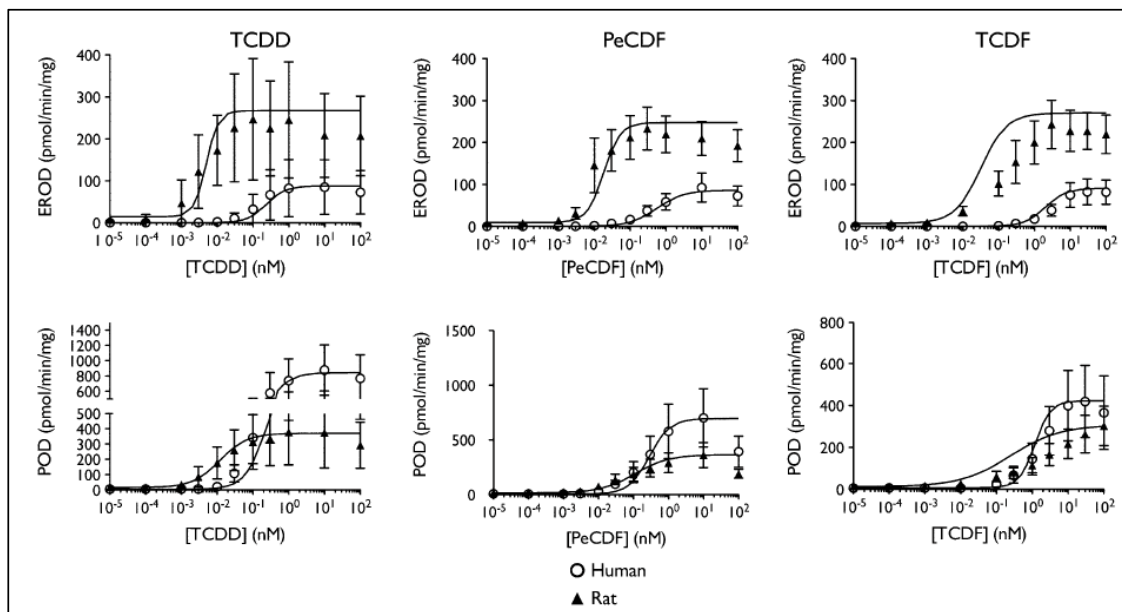


Figure A3-17. Comparison of rat and human dose-response for EROD and POD induction by the three congeners. Points are means and SDs of average of replicates from individual human and rat responses (Supplementary tables 1 and 2). Lines are Hill model fits using mean parameters from fits to averages of replicates from individuals (from: Budinsky *et al.*, 2010)

Although the studies of Budinsky *et al.* (2010) and Larsson *et al.* (2015) are probably the most demonstrative of the large species difference in TCDD and DL-PCBs in activating the human AhR, several additional studies provide additional strong support for this important species difference.

Carlson *et al.* (2009) did an exhaustive analysis of changes in gene expression in both human and rat liver cells following treatment with various doses of TCDD and PCB126. The summary of their results are shown in Figure A3-18.

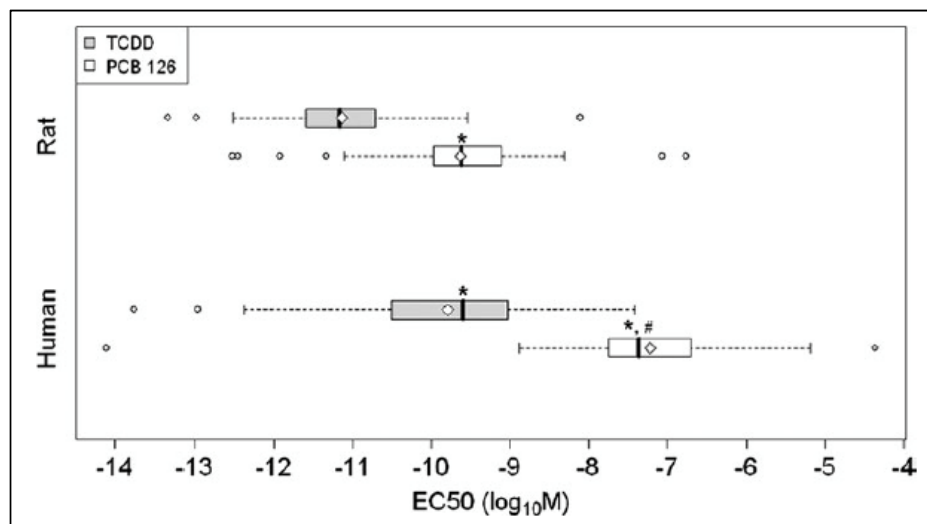


Figure A3-18. Standard Tukey boxplots summarizing the geometric mean species-specific EC50 MLEs for TCDD and PCB126. Redundant probe sets of the same gene were conservatively represented by the probe set with the lowest EC50 estimate. Note that the x-axis is in log10 Molar scale. Center black line of each box represents the median value, open diamonds are the geometric means, and hinges are the first and third quartiles. Whiskers extend to the most extreme data point no more than 1.5 times the interquartile range away from the box. Open circles represent outliers. Asterisks and pound (#) indicate statistical differences ($p \leq 0.05$) from unmarked EC50 estimates (from: Carlson *et al.*, 2009)

What is evident from these data is that: 1) the human response to TCDD is between 10-100 times less than that in the rat, and 2) the human response to PCB126 is at least 1,000 times less in humans than in rats, consistent with the more quantitative data reported by Budinsky *et al.* (2010) and Larsson *et al.* (2015).

Wahlang *et al.* (2014) looked at the ability of Aroclor 1260, as well as individual DL-PCB congeners present in 1260, to activate a variety of different human nuclear receptors. They found that Aroclor 1260 was a very weak activator of human AhR, requiring very high concentrations to achieve any notable increase, and the maximum increase, even at 20 mg/L, resulted in only a 4-fold increase in AhR activation (Figure A3-19A). When the Aroclor mixture was added to a potent AhR activator (10 uM benzo(a)anthracene), it had no significant additional activation (Figure A3-19B). Finally, when individual DL-PCB congeners were added into the system, only PCB126 had a biologically significant activation of the AhR (PCB138 had a modest response, but 118 had no response; Figure A3-19C).

While this study provides data only on human AhR and other nuclear receptors (PXR, CAR1,2,3, PPAR α , etc.) their results with the human AhR studies strongly support the conclusions of Larsson *et al.* (2015) that PCB126 is the only relevant PCB congener for human health risk assessments of DL-PCBs.

Silkworth *et al.* (2005) conducted studies comparing species differences in AhR response to Dioxin-like PCBs. They used primary hepatocytes from rat (female SD rats), rhesus monkey and from 5 different human livers. They conducted a dose-response analysis of Aroclor 1254, TCDD and PCB126, and measured both EROD and CYP1A1 mRNA. The results for EROD activity are shown in Figure A3-20. Analysis of EC₅₀ data demonstrated that human AhR response toward PCB126 was 0.00009 that of the rat AhR, or more than a thousand times less sensitive.

These findings are consistent with the findings of Larsson *et al.* (2015), who estimated that human AhR response to PCB126 is about 666-fold less sensitive than female SD rat liver.

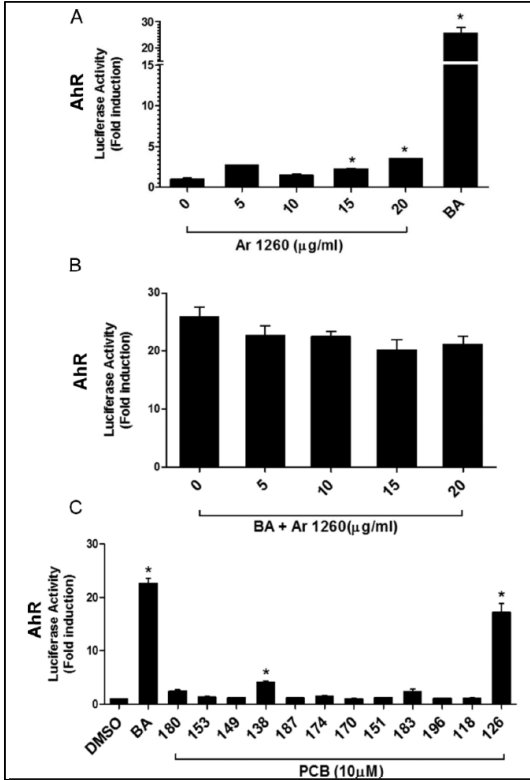


Figure A3-19. Aroclor 1260 Activation of human AhR (from: Wahlang *et al.*, 2014)

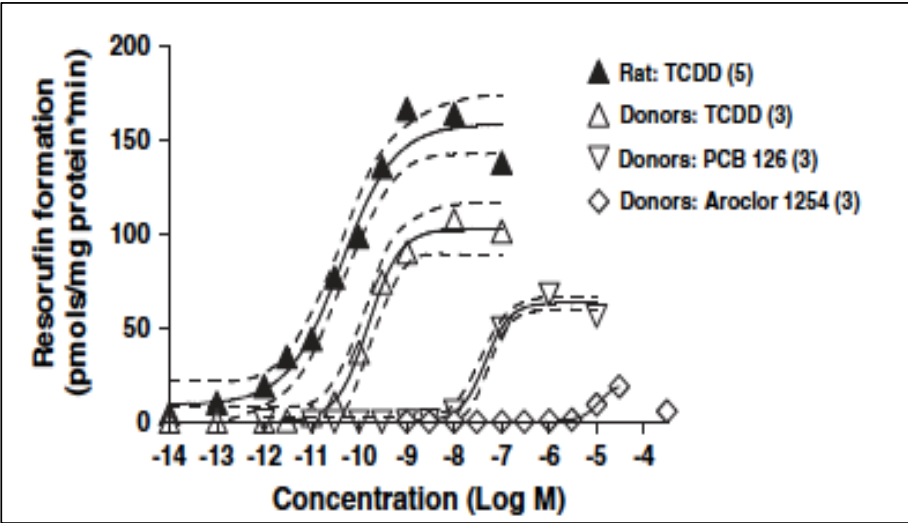


Figure A3-20. Comparison of human donor hepatocyte EROD response to the rat response to TCDD (Silkworth *et al.*, 2005)

These results further demonstrate the remarkable resistance of humans to PCB-mediated activation of human AhR (measured by CYP1A1 activity) and support a value of 666 as a reasonable, yet conservative, dose correction for differences between female SD rat liver and human AhR response to PCB126. The nearly

complete lack of human liver AhR response to Aroclor 1254 is consistent with the lack of AhR activity toward other DL-like PCBs (e.g., PCB118, 156 and 180) found by Larsson *et al.* (2015).

2. Determining a rat TEF from *in vivo* data, vs. *in vitro* cell based assays

The NTP studies on TCDD, PCB126 and PCB118 discussed in the previous section can also be used to determine Relative Effective Potencies (REPs) from *in vivo* tumor data, by calculating the approximate liver tissue concentration where 50% of animals developed liver tumors for TCDD, PCB126 and PCB118 (Figure A3-21). Based on *in vivo* rat tumor data (rather than *in vitro* cell assays such as those in Larsson *et al.* (2015)), PCB126 has a 'Toxic Equivalence Factor' (TEF) of 0.03, and PCB118 has a TEF of approximately 0.00004, for rat AhR activation.

These are quite close (within a factor of 3) to the TEFs calculated by Larsson *et al.* (2015), for rat AhR induction of EROD in rat liver hepatocytes (PCB126 rat TEF = 0.09, PCB118 rat TEF=0.00009).

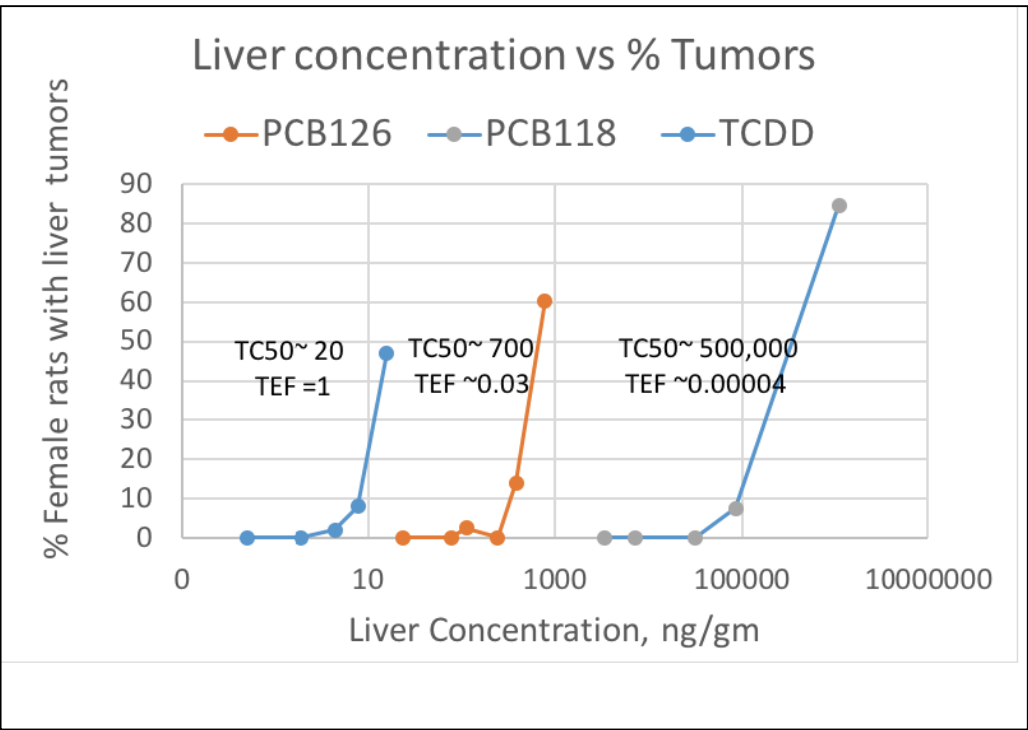


Figure A3-21. *In vivo* TEF estimation from NTP (2006b, 2006c, 2010) studies based on liver concentration and tumor response data

Thus, these data demonstrate that evidence-based human risk assessments based on experimental animal (primarily rat) data should be adjusted to use HUMAN AhR potency values, to estimate DL-PCB activation of AhR in humans.

VIII. Appendix 4: Immunotoxicology of PCBs

1. Introduction to immunotoxicology risk assessment

The immune system is composed of a series of balanced, complex, multicellular, and physiological mechanisms whose role is to preserve the integrity of the host. Functionally, the immune system distinguishes “self” tissues (organs and cells) from foreign (“non-self”) materials (e.g., bacteria, viruses, transformed cells) and then utilizes one or more of its highly specialized and complex systems to neutralize and/or eliminate the foreign materials. The immune system operates as a continuum, and any perturbation to the system by xenobiotics (i.e., foreign chemicals) may lead to altered immune competence. Changes leading to enhanced responsiveness (or failure to recognize self) can progress to autoimmune disease or hypersensitivity, while decreased ability to recognize (or neutralize/eliminate) foreign material can lead to immunosuppression and illness (Kaplan *et al.*, 2013).

The complexity of the immune system provides many potential targets that xenobiotics may affect. A brief overview of the immune system is presented to provide context for the numerous studies that have evaluated the immunotoxicity of PCBs.

The immune system consists of several lymphoid organs and many different cellular populations. Lymphoid organs are subdivided into two categories, primary and secondary, associated with the beginning/genesis and the amplification/protection phases of the immune system, respectively. Bone marrow and thymus are the primary lymphoid tissues because they support the production of mature B and T cells, respectively. In addition, the bone marrow originates pluripotent and self-renewing hematopoietic stem cells (HSCs) from which all other hematopoietic cells are derived.²⁵ Secondary lymphoid organs include the spleen and lymph nodes, which filter antigens from the blood and fluids surrounding body tissues, respectively. Within the secondary organs, filtered antigens contact and stimulate naïve T and B cells (i.e., cells that have never undergone antigen stimulation). Other secondary lymphoid tissues are associated with the skin, mucosal lamina propria, gut, bronchioles, and nasal cavity (Kaplan *et al.*, 2013).

Mammalian immunity is classified into two functional divisions:

- (1) Innate immunity – characterized as the first-line defense response. The magnitude of response is similar for first exposure and subsequent exposures/challenges. Key cell types: neutrophils, macrophages, natural killer (NK cells), and dendritic cells (DCs).
- (2) Acquired (adaptive) immunity – characterized by specificity and immunological memory. Speed and magnitude of response is greater for a secondary exposure/challenge than the first/initial exposure. Acquired immunity is classified into two subdivisions:
 - Humoral immunity – acquired immunity directly dependent on the production of antigen-specific antibody by B cells. It involves a coordinated interaction between antigen-presenting cells (APCs), T cells, and B cells.

²⁵ During gestation, HSCs are located in the embryonic yolk sac and fetal liver (Mikkola, H. K., and Orkin, S. H. (2006). The journey of developing hematopoietic stem cells. *Development* **133**(19), 3733-44.).

- Cell-mediated immunity (CMI) – acquired immunity that occurs without antibody involvement. It occurs through actions of effector cells (i.e., phagocytic cells, helper T cells, regulatory T cells, APCs, cytotoxic T cells, or T memory cells).

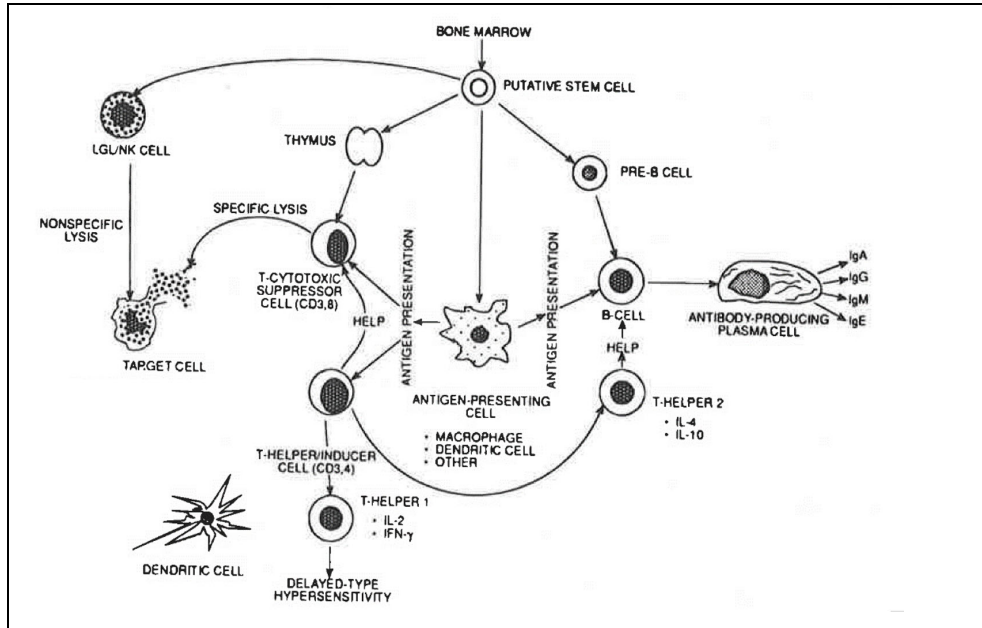


Figure A4-1. Selected cellular and biochemical components of the immune system (Derelanko and Hollinger, 1995)

The immune system encompasses a complex set of cellular and biochemical components. A graphical overview for selected cellular and biochemical aspects is provided in Figure A4-1; descriptions of the key components are below (Kaplan *et al.*, 2013):

- Antibodies are specific serum globulins, produced by B cells in response to the presence of an antigen.

Antibodies are defined functionally by the antigen with which they react and by their isotype (or subtype; see Table A4-1). For example, the IgM antibody directed against sheep red blood cells (SRBCs) is “anti-SRBC IgM.”

Antibodies of unknown specificity are referred to as immunoglobulin (e.g., serum immunoglobulin or serum IgM) until they can be defined by their specific antigen (e.g., anti-SRBC IgM).

Table A4-1. Antibody subtypes

Isotype	Biological properties
IgG	Complement fixation (selected subclasses) Crosses placenta
IgA	Secretory antibody
IgM	Complement fixation Efficient agglutination
IgD	Possible role in antigen-triggered lymphocyte differentiation
IgE	Allergic responses (mast-cell degranulation)

- An antigen is anything recognized by the immune system as “non-self.”
- Antigen presenting cells (APCs) are cells that take up antigen and processed for presentation to lymphocytes (to elicit an acquired immune response). These include dendritic cells (DCs), macrophages, and B cells.
- Antigen processing describes the process whereby accessory cells (i.e., APCs) take up, process, and present antigen to lymphocytes. Key events that occur following antigen encounter with T and B cells include:
 - Specific antigen recognition: T cells via MHC I or MHC II or B cells via Ig receptor,
 - Cellular activation and initiation of intracellular signaling cascades to release cytokines and other cellular mediators,
 - Proliferation (clonal expansion) of antigen-specific cells, and
 - Differentiation of antigen-stimulated lymphocytes into effector and memory cells.
- B cells (or B lymphocytes) are lymphocytes that develop in the bone marrow from hematopoietic stem cells (HSCs). B cells provide humoral immunity by antibody secretion; mature B cells differentiate into plasma cells, which results in a high serum concentration of specific antibody and establishment of immunological memory. Mature B cells become activated (and start proliferation) upon antigen binding to the surface immunoglobulin. After proliferation, the mature B cell undergoes differentiation into either a memory B cell or an antibody-forming cell (AFC or plaque forming cell; PFC) that actively secretes antigen-specific antibody.
- Cluster of differentiation (CD) is a series of cell surface markers (e.g., CD4, CD8) used to identify cell types and serve physiological roles.
- Complement is one consequence of antigen-antibody binding. A sequential pathway resulting in complement-mediated cell lysis.
- Dendritic cells (DCs) exist (in the absence of antigenic stimulation) in an immature state in most tissues, during which they can efficiently capture antigens. Maturation of DCs occurs in response to antigens; toll-like receptor (TLR) agonists (i.e., lipopolysaccharide [LPS] or cytokines such as GM-CSF or tumor necrosis factor, TNF- α). Mature DCs migrate to lymph nodes and present antigens to T cells.

DCs internalize antigen by phagocytosis, pinocytosis, or receptor-mediated endocytosis. Mature DCs then express high levels of both classes of MHC, which can stimulate both innate and acquired immune responses.

- Macrophages are phagocytic cells that can differentiate into tissue-specific macrophages that vary in extent of surface receptors, oxidative metabolism and expression of MHCII. Classically activated macrophages (M1) are pro-inflammatory and participate in antigen presentation; alternatively activated macrophages (M2) do not present antigen well, but are efficient in apoptotic (self-terminated, dead) cell removal.
- Major histocompatibility complex (MHC) is a group of cell surface markers of two classes (class I and class II; MHCI and MHCII). Major differences between the MHCI and MHCII pathways are:
 - MHCI is expressed by all nucleated cells; thus, MHCI processed and presented antigens are not limited to APCs.
 - MHCI antigenic peptides are usually smaller.
 - MHCI antigens to be processed are usually aberrantly expressed proteins, such as viral-associated proteins or mutated proteins.
 - Antigen processing and loading mechanisms for MHCI are slightly different than MHCII.
 - MHCI facilitates antigen presentation to CD8+ (cytotoxic) T cells (i.e., CMI), whereas MHCII facilitates presentation to CD4+ (helper) T cells (i.e., humoral immunity).
 - MHCI antigen processing and presentation is the major pathway by which virally infected cells are detected and killed by the acquired immune system.
- Neutrophils (also known as polymorphonuclear cells or PMNs) are phagocytic cells that can release various reactive oxygen species (ROS) to eliminate most microorganisms. Phagocytic activity is enhanced by the presence of complement and antibody deposited on the surface of the foreign target.
- Natural killer (NK) cells have two main functions: cytokine production and cytolysis. Predominant producers of interferon-gamma (IFN- γ), which helps mature DC. NK cells mediate both antibody-independent and antibody-dependent cellular cytotoxicity.
- Phagocytosis is a process of cellular ingestion; the cell membrane of a phagocyte engulfs other cells, bacteria, microorganisms, and debris. Once internalized, the phagocyte digests the engulfed material.
- T cells (or T lymphocytes) are lymphocytes that develop from pluripotent bone marrow stem cells that migrate to and develop in the thymus. Mature cells are transported to other lymphoid tissues (lymph nodes, spleen) where they can interact with B cells and accessory cells to stimulate antibody production or interact directly with antigen-bearing cells. T cells only recognize antigen when it is on the surface of a body cell. Accordingly, the T-cell receptor (TCR), which is uniquely produced through somatic recombination, recognizes antigen plus a surface marker (i.e., both classes of the MHC).

Activation of a mature T cell occurs upon binding of the TCR to MHC plus antigen; after proliferation, the mature T cell differentiates into either an effector cell or a memory cell. Subpopulations of T-cells include:

- Cytotoxic T cells (cytotoxic T lymphocyte; CTL) interact directly with antigen and destroy antigen-bearing cells; effector cell in CMI. Generally express CD8 (i.e., CD8+).
- Helper T cells (helper T lymphocytes; Th) help stimulate antibody production of B cells and certain other T cells. Generally express CD4 (i.e., CD4+). Further differentiate into phenotypes depending on cytokines present; two of which, Th1 and Th2, dictate whether CMI or humoral immunity will predominate, respectively (see Figure A4-1).

Th1 and Th2 cells express different extracellular cytokines that promote CMI and humoral defense against intracellular invaders (Th1 cells) or humoral defense against extracellular invaders (Th2 cells). Th1 and Th2 populations are not mutually exclusive; they negatively regulate each other so that a strong response of one inhibits response of the other.

- Regulatory T cells (Tregs; formerly suppressor T lymphocytes) are a small population of CD4+ cells that inhibit cell division or production of specific cytokines in certain other T and B cells.

Many different types of assays have been used to assess immune system status following exposure to different test articles. In general, these assays evaluate various aspects of immune response (e.g., antibody production or resistance to infection) of control animals compared to responses in groups of animals previously treated with different concentrations of test article. Toxicology testing to assess immunotoxicity in laboratory animals has been done through a tiered approach and when data from screening studies (which were defined differently for different organizations) indicate a chemical may be immunotoxic, functional tests would be then performed as part of an expanded tier in the same strain of animal (Luster *et al.*, 1988). The section below provides a brief overview of the types of assays that have been used to assess immune functions in animals following treatment by PCBs.

Gross immunopathology (organ weights and histopathology) assays

Histopathology changes and gross changes in lymphoid organs (i.e., weight and/or cellularity) are generally considered screening assays and supportive evidence for potential immunotoxicity and further investigation (Luster *et al.*, 1988).

Quantitation of specific cellular populations of B- and T-cells in the spleen is considered a more comprehensive evaluation of potential immune effects (Luster *et al.*, 1988).

Humoral (antibody-mediated) immunity assays

Humoral immunity is an acquired immunity directly dependent on the production of antigen-specific antibodies by B cells. It involves a coordinated interaction between antigen-presenting cells (APCs), T cells, and B cells. Measuring antibody production to specific antigens is a relatively comprehensive assessment of immune function because optimal antibody production to antigens requires a complex interaction between antigen presenting cells (APCs) (e.g., macrophages or dendritic cells), T-cells, B-cells, cytokines, functional major histocompatibility antigens, and cell surface receptors.

Methods to assess antibody production include hemagglutination, radial or linear immunodiffusion, immunoprecipitation, zone electrophoresis and immunoelectrophoresis, immunofluorescence, radioimmunoassay (RIA), plaque-forming cell (PFC) assay (also referred to as the antibody-forming cell or AFC) assay, and enzyme-linked immunosorbent assay (ELISA). Hemagglutination and immunodiffusion-precipitation-electrophoresis techniques are considered to lack optimal qualifications (i.e., sensitivity, reproducibility, simplicity, and measurement of parameters relevant to human and animal health) and do not correlate consistently with changes in host resistance to pathogens, tumor models, or autoimmunity (Burleson *et al.*, 1995). RIA and ELISA methods both measure levels of total concentration of specific antibodies in circulation. The PFC assay measures a slightly different parameter as it measures the number of specific antibody-producing plasma cells in a particular tissue (e.g., spleen, lymph node); it does not quantitate the level of antibody production or account for potential antibody production at sites not assessed. Both the ELISA and PFC methods results generally correlate with results from host resistance models (Burleson *et al.*, 1995).

Cell-mediated immunity assays

Cell-mediated immunity is an acquired immunity that occurs without antibody involvement. It is generally assessed using methods including the mixed lymphocyte reaction assay, cytotoxic T-lymphocyte assay, the delayed type hypersensitivity (DTH) assay, lymphocyte blastogenesis assays, and graft-vs-host response assays (International Programme on Chemical Safety, 1996; Luster *et al.*, 2003; Luster *et al.*, 1988). Briefly, the mixed lymphocyte reaction (MLR) assay mixes T-cells from the spleen or lymph nodes with allogenic (donor cells from same species of animal) stimulator cells and measures the response of T-cells to recognize the allogenic cells and proliferate. The cytotoxic T lymphocyte assay is a continuation of the MLR whereby spleen cells are removed and treated with stimulator cells (P815 mastocytoma cells with radiolabeled chromium). The percentage of radiolabeled chromium in the supernatant reflects the ability of TH1 cells to illicit cytotoxicity (and thereby immune competence). The delayed type hypersensitivity (DTH) assay is a TH1-dependent assessment that measures the magnitude of an inflammatory response at a sensitized compared to an unchallenged site. Lymphocyte blastogenesis assays measure blastogenesis and proliferation of sensitized T-cells in response to polyclonal activators such as the plant lectins concanavalin A (ConA) or phytohemagglutinin (PHA) (Pokeweed mitogen or PWM stimulates both T and B cells). Finally, graft-vs-host response/reactivity assays measure the ability of receptor animals (hosts) to immunologically respond to cell transfers (International Programme on Chemical Safety, 1996; Luster *et al.*, 2003; Luster *et al.*, 1988).

Nonspecific immunity assays

Nonspecific immune assays represent responses of the innate immune cells, including the natural killer (NK) cell activity assay and assays that assess macrophage activity. The natural killer (NK) cell assay measures splenic NK cell activity *in vitro* by culturing spleen cells with tumor cell lines known to be sensitive to NK-mediated cytotoxicity (e.g., YAC-1 or K562 for rat or primate NK evaluations, respectively). Target cells are radiolabeled so any lysed cells release radioactivity into the culture medium where it is measured. Macrophage activity is assessed through measuring their phagocytic or cytotoxic/killing ability (Luster *et al.*, 1988).

Host resistance assays

Host resistance assays assess potential altered susceptibility to infection or tumor cell challenge following exposure to a potential immunotoxicant. Assays include assessment of impaired host defenses through altering the resistance of the studied animal species to infection by various infectious agents, resistance to tumor growth following injection of tumor cells, and through altering concentrations associated with mortality of exposed populations compared to non-exposed populations (i.e., the LD50 or the lethal dose associated to kill 50% of the test population) (Luster *et al.*, 1988).

2. Hazard identification

A sequential search of PubMed was performed initially performed in March 2017 (and then repeated in August 2018, April 2019 and October 2019 to identify more recently published studies). Keywords used in each of the searching events are listed in Table A4-2. Together, these searches identified 601 unique citations. Resulting citation lists were refined through removal of those that met elimination criteria given in Table A4-2. This left a total of 260 articles that were further evaluated.²⁶

Table A4-2. PubMed literature searching strategy

Search strings	Elimination criteria
Aroclor AND immune	Written in foreign language
Aroclor AND immunotoxicity	Description of an analytical method without pertinent results
Aroclor AND immunotoxin	Evaluation of effects on wildlife/marine organisms
PCB AND immune	Did not assess immune effects of PCBs or Aroclors
PCB AND immunotoxicity	(e.g., study evaluated a different chemical with and without stimulation of liver enzymes by PCBs)
PCB AND immunotoxin	
PCBs AND immune	
PCBs AND immunotoxicity	
PCBs AND immunotoxin	
"Polychlorinated biphenyl" AND immune	
"Polychlorinated biphenyl" AND immunotoxicity	
"Polychlorinated biphenyl" AND immunotoxin	
"Polychlorinated biphenyls" AND immune	
"Polychlorinated biphenyls" AND immunotoxicity	
"Polychlorinated biphenyls" AND immunotoxin	

Studies were entered into the database when the study comprised a unique animal study (i.e., not in epidemiology study or a review); the test article studied included one or more PCBs and/or Aroclors; and the endpoints evaluated included at least one immune tissue or immune function assay. In total, data from 88 unique articles were entered into the database.

Each individual immune-related test consisted of a unique combination of test article, species, and endpoint; multiple tests were associated with most publications. Availability of unique information was the only criteria

²⁶ As applicable, additional publications identified through citations in the evaluated studies were also reviewed but the number of studies identified in this manner was not tracked.

for inclusion of individual tests; no filtration was performed to evaluate quality of included studies. The literature review resulted in identification of 403 individual immunotoxicity tests that represented 88 individual studies (publications).

Study design including test article identity, species, exposure route, and dosing frequency, duration, and concentration ranges were recorded for each study. In addition, if the study results found a concentration associated with a no observable effect level (NOAEL) for immunotoxicity, captured information included the highest NOAEL observed. The lowest observable effect level (LOAEL) for immunotoxicity was captured for each test when available. Results were sub categorized by type of immunotoxicity tests, including gross immunopathology (organ weight/histopathological) changes; humoral immunity; cell-mediated immunity; nonspecific [innate] immunity; host resistance; cell subset distribution changes; and a final category to capture other endpoints beyond those listed. A short description of all reviewed immunotoxicity tests are provided in Attachment 3.

As summarized in the table of studies provided in Attachment 3, studies investigating the potential immunotoxicity of PCBs began in the early 1970s and encompass numerous investigations across many different animal species, assays, and functional endpoints. Initial animal studies focused on assessing whether a hazard existed and thus focused on investigations with relatively high exposure concentrations and gross observations of organ size/weights and histopathology; these studies observed PCB-attributed immune-related effects including cellularity changes and diminished size (i.e., atrophy) of the thymus and spleen in PCB-treated animals. Assays of humoral immune function following PCB exposure observed changes to circulating lymphocytes, circulating immunoglobulin response, circulating antibody response, and decreased lymphocyte proliferation in response to T-cell mitogens (e.g., decreased plaque-forming cell [PFC] response to sheep red blood cells, SRBCs), although the concentration associated with observed effect levels (and non-observed effect levels) varied widely between studies, species, and congener. Although the potential for PCB-induced immunotoxicity has been most studied in rodents, results were often conflicting between studies, with some studies observing immune-related effect(s) while others did not reproduce the effect(s). Inconsistent experimental designs including variations in the number of test groups (e.g., one or multiple groups vs. controls) evaluated, concentration levels used, the route of exposure, and the exposure duration (e.g., single vs. multiple exposures) likely contributed to the large variability and lack of reproducibility between studies. In general, immune-related effects (gross effects to lymphoid organs) observed in laboratory animals treated with high concentrations of PCBs supported a potential hazard for immune-related effects.

3. Mode of action

As described below, scientific studies support that the mode of action for PCB-mediated immune effects is through activation of the AhR.

Much of the early research on the AhR focused on its hepatic expression and effects but soon spread to evaluation of non-hepatic tissue expression (and potential effects), which led to studies of AhR activity in immune organs. As summarized in a recent review article, the conclusion that that AhR activation adversely affected thymic function came first and was followed by many successive studies (in the 1980s) that demonstrated AhR-mediated suppression of the immune system included thymic atrophy, reduced cell-

mediated immunity, diminished humoral antibody responses and an overall reduction in resistance to infectious diseases (Kreitinger *et al.*, 2016).

The AhR's potential role in modulating the immune system has been extensively investigated. The AhR is commonly expressed in the immune system and is expressed in nearly all immune cell types (Kerkvliet, 2009). Functionally, the AhR can affect the transcriptional programs of Tregs and Th17, participate in the differentiation of Tr1 cells, and modulate the function of B cells, dendritic cells, and monocytes (Cella and Colonna, 2015; Wheeler *et al.*, 2017). Furthermore, AhR binds to dioxin response elements (DREs) or xenobiotic response elements (XREs). Many DREs/XREs affect immune-related genes such as cytokines IL10, IL21, IL17A/F, IL22, and ROR γ t, which have downstream effects on innate and adaptive immune cells (Tian *et al.*, 2015). Its importance is evident as immune system dysfunction is observed in AhR knockout mice. Although specific adverse effects in the AhR knockout are antigen-specific, downregulation of the immune system is the most commonly observed effect (Kerkvliet, 2009; Tian *et al.*, 2015).

2,3,7,8-tetrachlorodibenzodioxin (TCDD, dioxin) is the most potent and well-studied AhR ligand and has long been recognized as having immunosuppressant effects mediated through the AhR (Birnbaum and Tuomisto, 2000; Kerkvliet, 1995, 2009; Kreitinger *et al.*, 2016; Preston *et al.*, 1981; Sulentic and Kaminski, 2011; Tryphonas and Feeley, 2001). AhR-dependent responses to TCDD cause suppression of both the innate and adaptive immune systems with effects including thymic atrophy and dysfunction, humoral immunity suppression, cell mediated immunity suppression, and innate immunity (e.g., neutrophil, macrophage, NK cells, and dendritic cell-mediated) suppression (Birnbaum and Tuomisto, 2000; Kerkvliet, 2009; Sulentic and Kaminski, 2011; Tian *et al.*, 2015; Tryphonas and Feeley, 2001).

AhR-mediation in immunosuppressive effects has evolved over time with scientific evaluations of compounds structurally similar to TCDD. In particular, "[e]arly studies in the 1970's and 80's showed that various chlorinated congeners of dibenzo-p-dioxins, dibenzofurans and biphenyls produced similar immunosuppressive effects on the immune system of mice with a potency directly related to the affinity of the congener for binding to the [AhR]" (Kerkvliet, 2009).

Although the concentrations required to elicit immune effects with PCB exposures were higher, the resulting effects generally correlated with effects observed following TCDD exposures (i.e., suppressed cell-mediated immunity assessed through delayed hypersensitivity reactions, graft-versus-host responses, and decreased lymphocyte proliferation in response to T-cell mitogens), which strongly suggested immune effects attributed to PCB exposures are mediated through action of dioxin-like PCBs and their stimulation of the AhR (Silkworth and Loose, 1979a; Tryphonas and Feeley, 2001).

The AhR's role in modulating the immunotoxicity of PCBs was demonstrated through studies using different strains of mice that expressed AhR receptors with different structures and functions. Specifically, mutations in the DBA/2 mouse AhR gene result in expression of an AhR whose binding efficiency for ligands (e.g., TCDD and PCB77) is much less than the AhR expressed by C57BL/6 mice. The difference in ligand binding affinities cause functional differences in the ligand's ability to activate downstream events and the responsiveness to TCDD-mediated effects. Due to these functional differences, the C57BL/6 mice and DBA/2 mice are sometimes referred to as AhR-responsive and AhR non-responsive, respectively. One study evaluated the responses in each strain of mice following intraperitoneal injections of 0, 10, or 100 mg/kg PCB77 (dioxin-like) or PCB52 (non-dioxin like) given 2 days prior to and 2 days following intravenous inoculations of SRBCs.

Humoral immune response was evaluated using the anti-SRBC PFC assay, thymic atrophy, spleen cellularity, and organ weights. Increased PFCs, thymic atrophy and decreased relative spleen weights observed with treatment of the dioxin-like for PCB77 in C57BL/6 mice but not in DBA/2 mice. None of these effects were observed in either strain treated with the non-dioxin like PCB52 (Silkworth and Grabstein, 1982).

A second, similar study evaluated immune effects following intraperitoneal injections of 0, 10, or 100 mg/kg of dioxin-like PCB77 given 2 days before intravenous inoculations of SRBCs in mice with altered AhR phenotypes. Briefly, AhR responsive (BALB/cBy) and AhR nonresponsive (DBA/2) strains sharing the same major histocompatibility antigens were used. Bone marrow from the same or opposite AhR phenotype was used to reconstitute the bone marrow of lethally irradiated mice. Potential immune-related effects of PCB77 exposure were evaluated for normal and bone marrow chimeric mice using various tests including the anti-SRBC PFC assay, hemagglutination titer, spleen cellularity, and organ weights. The results demonstrated that the immunotoxicity was primarily determined through the AhR phenotype of the lymphoid tissue as PCB77 treatment suppressed the immune response only in mice with AhR sensitivity (Silkworth *et al.*, 1986).

One source reviewed indicated that non-AhR immunosuppressive effects of PCBs are mediated via PCB metabolism to “arene oxide intermediates capable of alkylating critical cellular macromolecules to form potentially toxic covalently bound substrate-macromolecule adducts” (Tryphonas and Feeley, 2001). However, the referenced publication (Preston *et al.*, 1981). is about PCB-induced hepatic tumors in the rat and does not mention metabolism, immune status, or reference any immunosuppressive effects (Preston *et al.*, 1981). I found no other data to support an adduct-mediated mechanism for PCB immunotoxicity.

As summarized above, TCDD-induced immunotoxicity has been well studied and science supports it is modulated through the AhR receptor. Multiple lines of evidence support immune effects following PCB exposures are also mediated by the AhR receptor. Studies have demonstrated PCB exposures generate similar immunosuppressive effects to those observed with TCDD; non-responsive AhR strains are not associated with immune-related effects of PCBs, and non-dioxin like PCB congeners are not associated with immunosuppressive effects.

4. Dose-response assessment

Dose-response is the fundamental tenet of toxicology (i.e., adverse response increases with dose), so high quality toxicology tests evaluate more than a single exposure concentration (generally three or more with a separate control group) with multiple administrations (for example one time per day over several days, weeks, months, or years).

As previously discussed, my hazard assessment of PCBs identified 403 immunotoxicity tests in 88 publications. This dataset was refined to include only those of typical laboratory animals (mice, rats, and monkeys) using oral routes of administration, which resulted in further evaluation of 180 tests that met the inclusion criteria (out of the originally identified 403 tests).

Studies must possess certain attributes before they can be used to ascertain whether a chemical can elicit a dose-response: they must include multiple test article administrations and compare multiple dose groups to a control group. Figure A4-2 graphically portrays the study designs of the 180 mouse, rat, or monkey oral administrations tests; 78% included multiple administrations; 60% included multiple dose groups; and 45% included both multiple dose groups and multiple test article administrations.

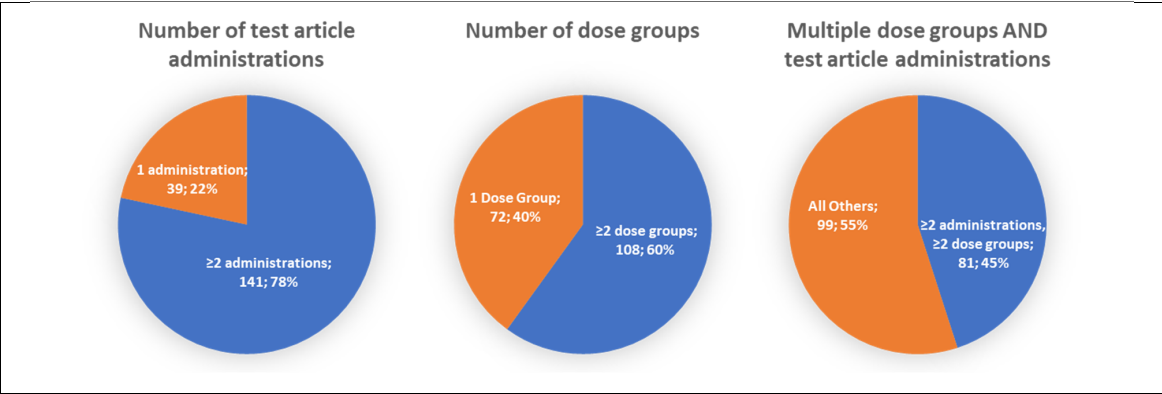


Figure A4-2. Quality of PCB and Aroclor immunotoxicity tests with oral route of administration

Of the 81 unique immunotoxicity tests that evaluated multiple dose groups and multiple administrations, 55 of those tests evaluated at least three dose groups (the minimum required for a dose-response assessment), and 35 of the 55 failed to show any immunotoxic effect any of the tested doses. In total, 20 tests remained for evaluation of dose-response. Of those remaining, some observed immune-related effects at the highest concentration tested only and thereby could not be used to assess whether a true dose-response relationship existed.

Significant dose-response trends were reported for both IgM and IgG following sheep red blood cell (SRBC) inoculation in Aroclor 1254 exposed monkeys (Tryphonas *et al.*, 1989; Tryphonas *et al.*, 1991a). Aroclor 1254 exposed monkeys also exhibited a significant dose-response trend in serum thymosin alpha-1 ($p = 0.0006$) but not an increased trend in serum complement activity ($p = 0.13$) (Tryphonas *et al.*, 1991b).

Statistical tests for a dose-response trend were conducted in none of the mouse studies. In one mouse study by Imanishi *et al.* (1980), the two highest administered doses of Kanechlor 500 tested (0.2 and 0.4 mg/kg) resulted in significantly increased mortality following Herpes simplex or Ectromelia virus infection than in control mice ($p < 0.05$) but there was no apparent difference between the effects induced by the 0.2 and 0.4 mg/kg treatments. Another mouse study conducted by Koller (1977) reported increased severity of splenic lesions in mice treated with PCBs and inoculated with Moloney Leukemia Virus (MLV) but results were not presented in a quantitative fashion. However, the authors also noted that MLV was not activated (i.e., no oncongenic effects were noted) in PCB-treated mice, suggesting that if any immunosuppression resulted from PCB exposure, it was insufficient to activate the virus. A recent study that dosed mice with PCB126 observed both decreased body weight gain and immune-related effects (e.g., reduced relative spleen and thymus weights) in all treatment groups (Du *et al.*, 2019).

Rat studies conducted by Miller *et al.* (1993) and Smialowicz *et al.* (1989) did not perform any statistical tests for a dose-response trend. In the Miller study, all tested doses of Aroclor 1254 (0.1 to 25 mg/kg) resulted in significantly elevated circulating corticosterone at 15 weeks post exposure, and in the Smialowicz study the two highest doses of Aroclor 1254 tested (10 and 25 mg/kg) resulted in significantly reduced NK cell activity.

Three studies conducted on rats by the NTP examined the carcinogenic effects of PCB118, PCB153, and PCB126 (NTP, 2006a, c, 2010). In the studies of PCB118 and 153, thymus and bone marrow effects,

respectively, were observed only at the highest administered dose. In the study of PCB126, thymus atrophy was observed at 0.175 µg/kg and increased in severity with increasing doses. This trend was not assessed for statistical significance.

As summarized above, most immunotoxicity studies that evaluated at least three doses with multiple oral administrations failed to find any immunotoxicity response in the treated animals. Of those remaining, monkey studies observed dose-responses for circulating antibodies. Single administrations of high doses of PCBs and related gross changes in immune organs (i.e., thymus and spleen) further support a dose-response relationship between PCB exposure and immunotoxicity (see summary of studies in Attachment 3).

5. Risk characterization

As described above, immunotoxicity following PCB exposure is dependent on AhR activation as was found with the cancer assessment (whose mode of action was also through activation of the AhR). In order to assess human risk related to any specific study, factors were needed to normalize the dose in animals to an equivalent dose in humans. These factors include considerations to account for (1) different abilities of different compounds (congeners or mixtures) to ability to activate the AhR in the tested animal species; (2) differences between AhR responsiveness (activation) in humans compared to the tested animal species; and (3) differences in animal to human body size differences.

Differences in activation of the AhR by different PCB congeners and TCDD has long been recognized. Toxic equivalency factors (TEFs) were established by the World Health Organization (WHO) for a list of 20 AhR ligands, including 12 dioxin-like PCBs based on data predominantly derived using rats and rat tissue. Because the highest affinity ligand for the AhR and the most potent at eliciting biologic responses in the rat is TCDD, its TEF was set to 1. TEFs for other chemicals were consensus estimates of the relative toxicity/potency of each chemical towards the AhR (relative to TCDD). The WHO last updated these values in 2006 (van den Berg *et al.*, 2006).

A publication nearly 10 years later by Larsson *et al.* (2015) reevaluated the relative effective potency for dioxin and various dioxin like PCBs *in vitro* among different species and provided robust evidence for establishing species-specific consensus toxicity factors (CTFs). Values derived by Larsson for rat, mouse, and human TEFs (or CTFs) are listed with the 2006 WHO consensus values in Table A4-3. Notable is that while Larsson's data indicate that the rat and mouse generally exhibit similar TEFs for dioxin-like PCBs (i.e., within an order of magnitude of each other), the human responsiveness is very different. Responsiveness differences between rats and humans were discussed extensively in the cancer section of this report and will not be repeated in this section. Extension of those same principles will occur in this section to the responsiveness of mice and monkeys relative to humans. The principles of extrapolation between species are the same and specific parameters and assumptions for those extrapolations are given in the text that follows. These principles form the foundation for calculations performed to characterize human equivalent doses from immunotoxicity tests of PCBs.

Table A4-3. TEFs for dioxin-like PCBs

Congener	2005 WHO consensus TEFs (van den Berg <i>et al.</i> , 2006)	Rat-specific consensus TEFs (Larsson <i>et al.</i> , 2015)	Mouse- specific TEFs (Larsson <i>et al.</i> , 2015) ²⁷	Human-specific consensus (Larsson <i>et al.</i> , 2015)
PCB77	0.0001	0.0004	0.002	0
PCB81	0.0003	0.0002	-	-
PCB126	0.1	0.09	0.05	0.003
PCB169	0.03	0.002	0.0005	0
PCB 74 ²⁸	-	0.000004	0.000002	0
PCB105	0.00003	0.00001	0.000002	0
PCB114	0.00003	0.00006	-	0
PCB118	0.00003	0.000009	0.000004	0
PCB123	0.00003	0.000009	-	0
PCB156	0.00003	0.00008	0.00004	0
PCB157	0.00003	0.00003	-	0
PCB167	0.00003	0.000007	0.000001	0
PCB189	0.00003	0.000007	-	0

Before human equivalent dose calculations were performed, immunotoxicity tests identified in Attachment 3 were refined to include those applicable for assessment of potential effects due to repeated exposures through fish consumption. Tests were refined to include only those with exposure regimes that included a minimum of two oral doses and those that studied typical laboratory animals (mice, rats, and monkeys).

In evaluating the resulting dataset, it was clear additional toxic equivalence (TEQ) values were necessary to evaluate the relative toxicity of tested mixtures of PCBs and different Aroclors.²⁹ TEQ values are derived by multiplying the concentration of each individual dioxin-like compound by its corresponding TEF. TEQs for mixtures are derived by summing the products for each dioxin-like compound in the mixture. Therefore, individual congener percentages of each Aroclor were necessary to derive the TEQ for each mixture. Table A4-4 provides available compositional congener analyses of different Aroclors (Frame *et al.*, 1996; Hong *et al.*, 1993; Kodavanti *et al.*, 2001; Rushneck *et al.*, 2004; Schulz *et al.*, 1989; Schwartz *et al.*, 1993; Takasuga *et al.*, 2005). Early analyses suffered from detection and/or quantitation deficits and were generally not as sensitive as later analyses and were not included in my calculations. The most recent and sensitive analysis by Rushneck *et al.* (2004) was used to estimate the abundance of congeners in the different Aroclors. Species-specific TEQs for Aroclors were derived using the Rushneck data combined with the species-specific TEFs presented in Table A4-3. Specifically, rat- and mouse-specific TEFs were derived using species-specific data presented by Larsson *et al.* (2015). The only species-specific TEF value available for monkeys was the TEQ for

²⁷ Mouse-specific TEF values estimated using concentrations estimated to elicit 20% of the maximum dioxin (TCDD) response (BMR20TCDD) for AhR induction using the luciferase assay in mouse liver cells (H1L6.1c2) per Larsson *et al.* (2015); TEF= (BMR20TCDD for TCDD; 0.011 nM) / (BMR20TCDD, specific PCB-congener, nM).

²⁸ Larsson *et al.*, 2015 was the first inclusion of PCB 74 as a dioxin-like congener.

²⁹ Aroclors were commercial mixtures of PCBs manufactured to specific chlorine content rather than to specific weight percentages of individual PCB congeners.

Aroclor 1254 determined by Silkworth Silkworth *et al.* (2005). Conservative assumptions were made for the remaining monkey TEFs, which were to assume they were equivalent to the WHO 2005 (van den Berg *et al.*, 2006) consensus TEF values (because those were generally the highest values amongst all species). All derived TEQs are presented in Table A4-5.

The next parameter considered for derivation of a human equivalent dose accounted for differences between AhR responsiveness (activation) in humans compared to the tested animal species. This animal to human dioxin AhR activation factor was derived as the ratio of the concentration associated with a response in humans divided by the concentration associated with the same response in the specific animal species as assessed by Silkworth *et al.* (2005) and Larsson *et al.* (2015). As summarized in Table A4-6, this resulted in animal to human TCDD AhR activation correction factors of 17, 26, and 10 for mice, rats, and monkeys, respectively. In other words, to correct for cross species extrapolation, it takes 17, 27, and 10 times more TCDD for the human AhR receptor to cause the same level of AhR activation in the mouse, rat and monkey.

The final parameter considered for derivation of a human equivalent dose accounted for body size differences between humans and animals. The species-specific dosimetric adjustment factor (DAF) adjusts for body size differences between test animals and humans using body surface areas (e.g., mg/m³ of surface area) of each. The body surface area relationship is approximated as the ratio of human to animal body weight (in kg) to the 0.75 (or $\frac{3}{4}$) power (US EPA, 2011d). Species-specific standard body weights (US EPA, 2005) were used with the average US adult body weight to derive DAFs were derived for mice, rats, and monkeys (Table A4-7).

Finally, the three adjustment factors defined for mouse, rat, and monkey studies were used to convert doses in animals into human equivalent doses. First, concentrations from immunotoxicity tests in animals (the lowest level that observed an immune-related effect: LOAEL and/or the highest concentration that did not observe an immune-related effect: NOAEL) were identified. Next, those doses were converted into dioxin-like equivalent concentrations through multiplication of the LOAEL or NOAEL dose by test article and species-specific TEFs and TEQs defined in Table A4-3 and Table A4-5 respectively. This calculation yields animal LOAEL or NOAEL doses in dioxin-equivalents.

Because both the animal to human body size and the AhR activation correction factors were the same across the three evaluated species, they were combined into a single value for each species: overall human adjustment factors were derived through division of animal to human dioxin AhR activation correction factors by applicable animal to human body size correction factors. This yielded overall animal to human adjustment factors of 2, 6, and 6 for mice, rats, and monkeys, respectively.

Human equivalent doses (HEDs) were then calculated for each immunotoxicity test evaluated through multiplication of the applicable species-specific animal to human adjustment factor by the previously calculated animal LOAEL or NOAEL doses (in dioxin-equivalents).

Risk characterization was performed through comparison of HEDs derived from immunotoxicity tests in animals to anticipated human exposure concentrations using a margin of exposure analysis, which is discussed in the next section.

Table A4-4. PCB congener analyses of Aroclors

PCB Congener (%) ^A														
Reference ^B	Lot	77	81	126	169	74 ^C	105	114	118	123	156	157	167	189
Aroclor 1016														
Schulz 1989	NS	ND	NA	ND	ND	0.89	ND	ND	ND	ND	ND	ND	ND	ND
Hong 1993	NS	0.0082	ND	ND	ND	NA	0.0062	ND	0.0046	0.0003	ND	ND	0.0043	ND
Frame 1996	A2 ^D	ND	ND	ND	ND	0.33	0 ^E	ND	ND	ND	ND	ND	ND	ND
	S2 ^D	ND	ND	ND	ND	0.33	ND	ND	ND	ND	ND	ND	ND	ND
Rushneck 2004	129	0.00409	0.000196	0.000056	0.000013	NA	0.00695	0.000603	0.0110	0.000472	0.000372	0.000103	0.000110	0.000012
Aroclor 1221														
Hong 1993	NS	0.0074	ND	ND	ND	NA	0.0068	0.0006	0.0064	0.0012	0.0026	ND	0.0014	ND
Frame 1996	A1 ^D	0.01	ND	ND	ND	0.12	0.05	ND	0.08	ND	ND	ND	ND	ND
Rushneck 2004	A7080365	0.00126	0.000051	0.000028	ND	NA	0.00559	0.000404	0.00881	0.000333	0.000749	0.000146	0.000252	0.000117
Aroclor 1232														
Hong 1993	NS	0.1200	0.0118	0.0009	ND	NA	0.2470	0.0160	0.1220	0.0100	0.0150	0.0042	0.0042	0.0009
Frame 1996	A1.5 ^D	0.17	0 ^E	ND	ND	0.92	0.22	0.02	0.29	ND	ND	ND	ND	ND
	G1.5 ^D	0.16	ND	ND	ND	0.92	0.21	0.01	0.28	ND	ND	ND	ND	ND
Rushneck 2004	A7080363	0.2150	0.0111	0.00210	ND	NA	0.3030	0.0248	0.4460	0.0164	0.00907	0.00220	0.00324	0.000436
Aroclor 1242														
Schulz 1989	NS	0.45	NA	ND	ND	2.17	0.86	ND	1.62	ND	0.09	ND	ND	ND
Hong 1993	NS	0.2670	0.0212	0.0029	ND	NA	0.5380	0.0350	0.2300	0.0240	0.0241	0.0076	0.0061	ND
Schwartz 1993														
GCMS	NS	0.2200	0.0140	0.0020	<0.0001 ^F	NA	0.3300	0.0300	0.5000	0.0100	0.0042	0.0017	0.0023	0.0005
GCECD		0.1700	0.0159	0.0016	<0.0012 ^F	NA	0.2670	0.0328	0.3820	0.0063	0.0036	0.0019	0.0020	<0.0007 ^F
Frame 1996	A3 ^D	0.27	0 ^E	ND	ND	1.83	0.52	0.05	0.78	0.03	0.02	ND	ND	ND
	G3 ^D	0.33	0.01 ^G	ND	ND	1.76	0.37	0.03	0.51	0.02	ND	ND	ND	ND
	S3B ^D	0.33	0.01 ^G	ND	ND	1.84	0.52	0.05	0.69	0.03	ND	ND	ND	ND
Rushneck 2004	01141	0.2590	0.0156	0.00336	^H	NA	0.4840	0.0443	0.6980	0.0277	0.0255	0.00709	0.00807	0.000453

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Table A4-4. PCB congener analyses of Aroclors

Reference ^B	PCB Congener (%) ^A													
	Lot	77	81	126	169	74 ^C	105	114	118	123	156	157	167	189
Aroclor 1248														
Hong 1993	NS	0.3000	0.0270	0.0100	ND	NA	1.8000	0.1160	0.7900	0.0650	0.0560	0.0165	0.0300	0.0018
Schwartz 1993														
GCMS	NS	0.3500	0.0260	0.0074	<0.0002 ^F	NA	1.7000	0.1400	2.5000	0.0500	0.0610	0.0170	0.0280	0.0017
GCECD		0.2990	0.0305	0.0038	<0.0002 ^F	NA	1.3600	0.1623	1.9900	0.0280	0.0387	0.0101	0.0185	0.0011
Frame 1996	A3.5 ^D	0.41	0.01	0 ^E	ND	3.14	1.60	0.12	2.29	0.07	0.06	0 ^E	0 ^E	ND
	G3.5 ^D	0.52	0.02	0 ^E	ND	4.67	1.45	0.12	2.35	0.08	0.04	0 ^E	0 ^E	ND
Rushneck 2004	A7090364	0.4440	0.0221	0.00980	0.000021	NA	1.7300	0.132	2.4200	0.0806	0.0654	0.0171	0.0207	0.00110
Aroclor 1254														
Schulz 1989	NS	ND	NA	ND	ND	0.78	3.83	ND	6.39	0.81	1.62	ND	0.21	ND
Hong 1993	NS	0.1550	0.0174	0.0250	ND	NA	6.9000	0.4150	2.7400	0.2170	0.8360	0.2880	0.1800	0.0250
Schwartz 1993														
GCMS	NS	0.0220	0.0010	0.0033	<0.0001 ^F	NA	3.0000	0.1700	7.8000	0.0400	0.3900	0.1800	0.2400	0.0250
GCECD		0.0200	<0.0004 ^F	0.0088	<0.0003 ^F	NA	3.2100	0.2460	7.5800	0.0560	0.7610	0.3410	0.4390	0.0268
Frame 1996	A4 ^{D,I}	0.20	0 ^E	0.02	ND	2.19	7.37	0.50	13.59	0.32	1.13	0.30	0.35	0 ^E
	G4 ^{D,I}	0.03	ND	0 ^E	ND	0.84	2.99	0.18	7.35	0.15	0.82	0.19	0.27	0.01
Kodavanti 2001	124-191 ^K	0.001	0.001	0.017	0.001	0.436	5.100	0.005 ^L	12.700	0.057	0.480	0.036	ND	ND
	6024 ^M	2.720	0.028	0.324	0.002	2.347	13.000	0.078 ^L	12.400	0.214	5.100	2.630	ND	ND
Rushneck 2004	124-191	0.0174	0.00164	0.00373	0.000081	NA	3.3800	0.1930	7.8900	0.1150	0.8440	0.1870	0.3100	0.0246
Aroclor 1260														
Schulz 1989	NS	ND	NA	ND	0.05	ND	0.07	ND	0.57	ND	0.88	0.14	0.26	0.11
Hong 1993	NS	0.0210	0.0025	0.0024	ND	NA	0.0450	0.0030	0.3900	0.0440	0.3130	0.0200	0.1560	0.1060
Schwartz 1993														
GCMS	NS	0.0060	<0.0005 ^F	<0.0003 ^F	<0.0001 ^F	NA	0.0280	0.0012	0.5000	<0.0002 ^F	0.2400	0.0260	0.1600	0.0960
GCECD		<0.0061 ^F	<0.0055 ^F	<0.0052 ^F	<0.0042 ^F	NA	0.0245	0.0028	0.4470	<0.0020 ^F	0.2940	NA ^N	0.1900	0.0885

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Table A4-4. PCB congener analyses of Aroclors

Reference ^B	PCB Congener (%) ^A													
	Lot	77	81	126	169	74 ^C	105	114	118	123	156	157	167	189
Frame 1996	A5 ^D	ND	ND	ND	ND	0.05	0.22	0 ^E	0.51	ND	0.53	0.02	0.20	0.08
	S5 ^D	ND	ND	ND	ND	0.05	0.21	ND	0.50	ND	0.54	0.02	0.20	0.11
	G5 ^D	ND	ND	ND	ND	0.04	0.23	0 ^E	0.45	ND	0.50	0.02	0.17	0.12
Rushneck 2004	023-150D	0.00338	0.000333	0.000213	ND	NA	0.0434	0.00170	0.5610	0.000502	0.4860	0.0252	0.1990	0.1290
Aroclor 1262														
Hong 1993	NS	0.0110	0.0011	0.0032	ND	NA	0.0760	0.0045	0.1510	0.0365	0.0730	0.0094	0.0284	0.0420
Frame 1996	A6 ^D	ND	ND	ND	ND	0.06	0.18	ND	0.17	ND	0.14	0 ^E	0.02	0.03
	G6 ^D	ND	ND	ND	ND	0.04	ND	ND	0.14	ND	0.18	0 ^E	0.05	0.04
Rushneck 2004	106-262	0.00846	0.000463	0.000228	0.000040	NA	0.0764	0.00460	0.1980	0.00278	0.0946	0.00638	0.0278	0.0451
Aroclor 1268														
Hong 1993	NS	0.0130	0.0009	0.0005	ND	NA	0.0234	0.0012	0.0182	0.0010	0.0024	0.0064	0.0019	0.0005
Rushneck 2004	A7080368	0.00361	0.000135	0.000176	ND	NA	0.0107	0.000586	0.0101	0.000324	0.00176	^H	0.000496	0.000440
Kanechlor 500														
Takasuga 2005	NS	0.075	0.0024	0.0022	0.00005	NA	2.1	0.12	5.4	0.10	0.88	0.19	0.30	0.031

^A Abbreviations: NA, congener not analyzed; ND, not detected; NS, not specified.

^B Frame, G. M., Cochran, J. W., and Bowadt, S. S. (1996). Complete PCB congener distributions for 17 aroclor mixtures determined by 3 HRGC systems optimized for comprehensive, quantitative, congener-specific analysis. *J High Resol Chromat* **19**, 657-668, Hong, C. S., Bush, B., Xiao, J., and Qiao, H. (1993). Toxic potential of non-ortho and mono-ortho coplanar polychlorinated biphenyls in Aroclors, seals, and humans. *Arch Environ Contam Toxicol* **25**(1), 118-23, Kodavanti, P. R., Kannan, N., Yamashita, N., Derr-Yellin, E. C., Ward, T. R., Burgin, D. E., Tilson, H. A., and Birnbaum, L. S. (2001). Differential effects of two lots of Aroclor 1254: congener-specific analysis and neurochemical end points. *Environ Health Perspect* **109**(11), 1153-61, Rushneck, D. R., Beliveau, A., Fowler, B., Hamilton, C., Hoover, D., Kaye, K., Berg, M., Smith, T., Telliard, W. A., Roman, H., *et al.* (2004). Concentrations of dioxin-like PCB congeners in unweathered Aroclors by HRGC/HRMS using EPA Method 1668A. *Chemosphere* **54**(1), 79-87, Schulz, D. E., Petrick, G., and Duinker, J. C. (1989). Complete characterization of polychlorinated biphenyl congeners in commercial Aroclor and Clophen mixtures by multidimensional gas chromatography-electron capture

detection. *Environmental Science & Technology* **23**(7), 852-859, Schwartz, T. R., Tillitt, D. E., Feltz, K. P., and Peterman, P. H. (1993). Determination of mono- and non-o,o'-chlorine substituted polychlorinated biphenyls in Aroclors and environmental samples. *Chemosphere* **26**(8), 1443-1460, Takasuga, T., Kumar, K. S., Noma, Y., and Sakai, S. (2005). Chemical characterization of polychlorinated biphenyls, -dibenzo-p-dioxins, and -dibenzofurans in technical Kanechlor PCB formulations in Japan. *Arch Environ Contam Toxicol* **49**(3), 385-95.

^c Larsson *et al.*, 2015 was the first inclusion of PCB 74 as a dioxin-like congener.

^d A-designated lot #s were obtained from AccuStandard; G-designated lot #s were obtained from Monsanto Corp. and were used as secondary HRGC calibration standards by GE Corporate R&D; and S-designated lots #s were obtained from Supelco (Frame *et al.*, 1996).

^e No value reported for weight percent due to uncertain accuracy for peaks near background noise levels (<0.005%).

^f Less than detection limit.

^g Values calculated between 0.015 and 0.005 were rounded to 0.1 (Frame *et al.*, 1996).

^h Peak detected did, but not did not meet identification criteria.

ⁱ Lot A4 of Aroclor 1254 was described by Frame *et al.* (1996) as "atypical" and no longer distributed [this lot most likely represents the later, 2-stage production process].

^j Lot G4 of Aroclor 1254 (obtained from Monsanto) was described by Frame *et al.* (1996) to contain a distribution of PCBs closer to the majority of other lots of Aroclor 1254.

^k Lot 124-191 of Aroclor 1254 represents the typical PCB congener distribution [characteristic of the production process prior to 1974] (Kodavanti *et al.*, 2001).

^l Value is sum of 114, 122, and 131.

^m Lot 6024 of Aroclor 1254 "was traced back to Monsanto lot KI-6024 and represents the late (1974-1976) production procedure" (Kodavanti *et al.*, 2001). This agrees with Mayes (1998), which indicated the lot represented the later production process. The late production process used two stages. In the first stage, biphenyl was chlorinated to 42% chlorine content by weight as for Aroclor 1242 production and then fractionated to give a distillate sold as Aroclor 1016. In the second stage, residue from the distillate obtained in the first stage was further chlorinated to 54% chlorine by weight, greatly increasing the levels on non-ortho and mono-ortho congeners with high TEF values (Kodavanti *et al.*, 2001).

ⁿ Interference precluded accurate determination.

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Table A4-5. TEQs for Aroclors

ID	Rat dioxin correction (TEQ) factor	Source(s) for rat congener composition & TEQ	Mouse dioxin correction (TEQ) factor	Source(s) for mouse congener composition & TEQ	Monkey dioxin correction (TEQ) factor	Source(s) for monkey congener composition & TEQ
Aroclor 1016	7.00E-08	Rushneck, 2004; Larsson, 2015	1.10E-07	Rushneck, 2004; Larsson, 2015	7.00E-08	Rushneck, 2004; van Den Berg, 2006
Aroclor 1221	3.30E-08	Rushneck, 2004; Larsson, 2015	4.00E-08	Rushneck, 2004; Larsson, 2015	3.40E-08	Rushneck, 2004; van Den Berg, 2006
Aroclor 1232	2.90E-06	Rushneck, 2004; Larsson, 2015	5.40E-06	Rushneck, 2004; Larsson, 2015	2.60E-06	Rushneck, 2004; van Den Berg, 2006
Aroclor 1242	4.30E-06	Rushneck, 2004; Larsson, 2015	6.90E-06	Rushneck, 2004; Larsson, 2015	4.10E-06	Rushneck, 2004; van Den Berg, 2006
Aroclor 1248	1.10E-05	Rushneck, 2004; Larsson, 2015	1.40E-05	Rushneck, 2004; Larsson, 2015	1.20E-05	Rushneck, 2004; van Den Berg, 2006
Aroclor 1254	5.40E-06	Rushneck, 2004; Larsson, 2015	2.90E-06	Rushneck, 2004; Larsson, 2015	1.80E-04	Silkworth, 2005
Aroclor 1260	6.80E-07	Rushneck, 2004; Larsson, 2015	3.90E-07	Rushneck, 2004; Larsson, 2015	6.50E-07	Rushneck, 2004; van Den Berg, 2006
Aroclor 1262	3.50E-07	Rushneck, 2004; Larsson, 2015	3.30E-07	Rushneck, 2004; Larsson, 2015	3.90E-07	Rushneck, 2004; van Den Berg, 2006
Aroclor 1268	1.80E-07	Rushneck, 2004; Larsson, 2015	1.60E-07	Rushneck, 2004; Larsson, 2015	1.90E-07	Rushneck, 2004; van Den Berg, 2006

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Table A4-6. Correction factors for animal to human dioxin AhR activation differences

Species	Animal to human dioxin AhR activation correction factor	Basis
Rat	26	Larsson <i>et al.</i> (2015): benchmark response for 20% of maximal EROD induction for TCDD in primary hepatocytes. BMR20TCDD ratio: (humans, 0.11 nM) (rats, 0.0042 nM)
Monkey	10	Silkworth <i>et al.</i> (2005): EC50 for EROD activity for TCDD in primary hepatocytes. EC50 ratio: (humans, 0.29 nM) (monkeys, 0.028 nM)
Mouse	17	Larsson <i>et al.</i> (2015): benchmark response for 20% of maximal AhR induction (Luciferase) for TCDD in human HepG2-AZ-AhR cells and mouse H1L6.1c2 cells BMR20TCDD ratio: (humans, 0.19 nM) (mice, 0.011 nM)

*: Cell line developed for CALUX (Chemically Activated Luciferase eXpression) bioassay. CALUX is used for rapid screening of samples for the presence of dioxin/TCDD-like compounds and AhR agonists/antagonists. Characteristics: Acts through the activation of a luciferase gene under the control of dioxin/TCDD-response elements (DREs). Transfected with: UniProtKB; P08659; Firefly luciferase.

Table A4-7. Species-specific animal to human DAFs (body size adjustment factors)

Animal species	Average animal body weight (kg) (US EPA, 2005)	Average human body weight (kg) (US EPA, 2011a)	Animal to human DAF
Mouse	0.025	80	7.5
Rat	0.25	80	4.2
Monkey	9	80	1.7

6. Margin of exposure analysis

Table A4-8. MOEs for NOAELs of immunotoxicity in PCB studies of mice, rats and monkeys with 2 or more oral administrations

Category	Endpoint	Species (sex)	Test article	Dosing parameters	Animal NOAEL dose	HED, dioxin-equivalents	MOE	Reference
Humoral immunity	Circulating gamma globulin (electrophoresis)	Monkey (female)	Aroclor 1248	Route: oral (diet) Duration: 1/d; ~16 mo	0.1000 mg/kg-d	0.0000072 mg/kg-d	885,000	(Thomas and Hinsdill, 1978)
Humoral immunity	Circulating IgM response (SRBC antigen; hemolysis)	Monkey (female)	Aroclor 1248	Route: oral (diet) Duration: 1/d; ~16 mo	0.1000 mg/kg-d	0.0000072 mg/kg-d	885,000	(Thomas and Hinsdill, 1978)
Organ weight, histopath	Organ weight, histopath	Monkey (not specified [both])	Aroclor 1248	Route: in utero and nursing Duration: 1/d; Discontinued treated diet of mothers 1 yr before mating	0.1000 mg/kg-d	0.0000072 mg/kg-d	885,000	(Allen <i>et al.</i> , 1980)
Cell mediated immunity	Lymphocyte blastogenesis, T cell mitogen(s) ConA, PHA	Monkey (both)	Mixture (major PCBs in Canadian human milk)	Route: oral (in corn oil) Duration: 1/d; 20 wks exposure, 66 wks total (w/ follow-up)	0.0075 mg/kg-d	0.0000072 mg/kg-d	885,000	(Arnold <i>et al.</i> , 1999)

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Category	Endpoint	Species (sex)	Test article	Dosing parameters	Animal NOAEL dose	HED, dioxin-equivalents	MOE	Reference
Cell subset distributions	Circulating cells	Monkey (both)	Mixture (major PCBs in Canadian human milk)	Route: oral (formula) Duration: 1/d; 20 wks exposure, 66 wks total (w/ follow-up)	0.0075 mg/kg-d	0.0000072 mg/kg-d	885,000	(Arnold <i>et al.</i> , 1999)
Cell subset distributions	Circulating cells	Monkey (males)	Mixture (major PCBs in Canadian human milk)	Route: oral (in corn oil) Duration: 1/d; 20 wks exposure, 66 wks total (w/ follow-up)	0.0075 mg/kg-d	0.0000072 mg/kg-d	885,000	(Arnold <i>et al.</i> , 1999)
Humoral immunity	Circulating IgG response (SRBC antigen; hemagglutination)	Monkey (males)	Mixture (major PCBs in Canadian human milk)	Route: oral (in corn oil) Duration: 1/d; 20 wks exposure, 66 wks total (w/ follow-up)	0.0075 mg/kg-d	0.0000072 mg/kg-d	885,000	(Arnold <i>et al.</i> , 1999)
Humoral immunity	Lymphocyte blastogenesis, B,T cell mitogen(s) PWM	Monkey (both)	Mixture (major PCBs in Canadian human milk)	Route: oral (in corn oil) Duration: 1/d; 20 wks exposure, 66 wks total (w/ follow-up)	0.0075 mg/kg-d	0.0000072 mg/kg-d	885,000	(Arnold <i>et al.</i> , 1999)
Nonspecific immunity	NK cell activity	Monkey (both)	Mixture (major PCBs in Canadian human milk)	Route: oral (formula) Duration: 1/d; 20 wks exposure, 66 wks total (w/ follow-up)	0.0075 mg/kg-d	0.0000072 mg/kg-d	885,000	(Arnold <i>et al.</i> , 1999)
Nonspecific immunity	NK cell activity	Monkey (males)	Mixture (major PCBs in Canadian human milk)	Route: oral (in corn oil) Duration: 1/d; 20 wks exposure, 66 wks total (w/ follow-up)	0.0075 mg/kg-d	0.0000072 mg/kg-d	885,000	(Arnold <i>et al.</i> , 1999)

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Category	Endpoint	Species (sex)	Test article	Dosing parameters	Animal NOAEL dose	HED, dioxin-equivalents	MOE	Reference
Humoral immunity	Circulating IgG response (Tetanus antigen; hemagglutination)	Monkey (female)	Aroclor 1248	Route: oral (diet) Duration: 1/d; ~16 mo	0.2000 mg/kg-d	0.0000144 mg/kg-d	1,770,000	(Thomas and Hinsdill, 1978)
Humoral immunity	Circulating IgG response (SRBC antigen; hemagglutination)	Monkey (females)	Aroclor 1254	Route: oral (gelatin capsule) Duration: 1/d; 55 mo	0.0200 mg/kg-d	0.0000216 mg/kg-d	2,654,000	(Tryphonas <i>et al.</i> , 1991a)
Cell mediated immunity	Lymphocyte blastogenesis, T cell mitogen(s) ConA, PHA	Monkey (females)	Aroclor 1254	Route: oral (gelatin capsule) Duration: 1/d; 23 mo	0.0800 mg/kg-d	0.0000864 mg/kg-d	10,618,000	(Tryphonas <i>et al.</i> , 1989)
Cell mediated immunity	Lymphocyte blastogenesis, T cell mitogen(s) ConA, PHA	Monkey (females)	Aroclor 1254	Route: oral (gelatin capsule) Duration: 1/d; 55 mo	0.0800 mg/kg-d	0.0000864 mg/kg-d	10,618,000	(Tryphonas <i>et al.</i> , 1991a)
Cell mediated immunity	Mixed Lymphocyte reaction	Monkey (females)	Aroclor 1254	Route: oral (gelatin capsule) Duration: 1/d; 55 mo	0.0800 mg/kg-d	0.0000864 mg/kg-d	10,618,000	(Tryphonas <i>et al.</i> , 1991a)
Cell subset distributions	Circulating cells	Monkey (females)	Aroclor 1254	Route: oral (gelatin capsule) Duration: 1/d; 55 mo	0.0800 mg/kg-d	0.0000864 mg/kg-d	10,618,000	(Tryphonas <i>et al.</i> , 1991a)
Circulating factors	Hydrocortisone	Monkey (females)	Aroclor 1254	Route: oral (gelatin capsule) Duration: 1/d; 22 mo	0.0800 mg/kg-d	0.0000864 mg/kg-d	10,618,000	(Loo <i>et al.</i> , 1989)
Circulating factors	Hydrocortisone	Monkey (females)	Aroclor 1254	Route: oral (gelatin capsule) Duration: 1/d; 23 mo	0.0800 mg/kg-d	0.0000864 mg/kg-d	10,618,000	(Tryphonas <i>et al.</i> , 1989)
Circulating factors	Hydrocortisone	Monkey (females)	Aroclor 1254	Route: oral (gelatin capsule) Duration: 1/d; 55 mo	0.0800 mg/kg-d	0.0000864 mg/kg-d	10,618,000	(Tryphonas <i>et al.</i> , 1991a)

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Category	Endpoint	Species (sex)	Test article	Dosing parameters	Animal NOAEL dose	HED, dioxin-equivalents	MOE	Reference
Humoral immunity	Circulating antibody response (pneumococcal antigens; RIA)	Monkey (females)	Aroclor 1254	Route: oral (gelatin capsule) Duration: 1/d; 55 mo	0.0800 mg/kg-d	0.0000864 mg/kg-d	10,618,000	(Tryphonas <i>et al.</i> , 1991a)
Humoral immunity	Circulating IgG, IgM, IgA (ELISA)	Monkey (females)	Aroclor 1254	Route: oral (gelatin capsule) Duration: 1/d; 23 mo	0.0800 mg/kg-d	0.0000864 mg/kg-d	10,618,000	(Tryphonas <i>et al.</i> , 1989)
Humoral immunity	Lymphocyte blastogenesis, B,T cell mitogen(s) PWM	Monkey (females)	Aroclor 1254	Route: oral (gelatin capsule) Duration: 1/d; 55 mo	0.0800 mg/kg-d	0.0000864 mg/kg-d	10,618,000	(Tryphonas <i>et al.</i> , 1991a)
Nonspecific immunity	NK cell activity	Monkey (female)	Aroclor 1254	Route: oral (gelatin capsule) Duration: 1/d; 55 mo	0.0800 mg/kg-d	0.0000864 mg/kg-d	10,618,000	(Tryphonas <i>et al.</i> , 1991b)
Other	IL-1 release, monocytes +LPS	Monkey (females)	Aroclor 1254	Route: oral (gelatin capsule) Duration: 1/d; 55 mo	0.0800 mg/kg-d	0.0000864 mg/kg-d	10,618,000	(Tryphonas <i>et al.</i> , 1991a)
Humoral immunity	Circulating IgG, IgM, IgA (immunoplates [ELISA])	Monkey (females)	Aroclor 1254	Route: oral (juice) Duration: 5/w; 12-13 mo	0.2000 mg/kg-d	0.0002160 mg/kg-d	26,544,000	(Tryphonas <i>et al.</i> , 1986)
Humoral immunity	Circulating IgG, IgM, IgA (immunoplates [ELISA])	Monkey (females)	Aroclor 1254	Route: oral (juice) Duration: 5/w; 27-28 mo	0.2000 mg/kg-d	0.0002160 mg/kg-d	26,544,000	(Tryphonas <i>et al.</i> , 1986)
Humoral immunity	Circulating IgM response (SRBC antigen; hemolysis)	Monkey (females)	Aroclor 1254	Route: oral (juice) Duration: 5/w; 12-13 mo	0.2000 mg/kg-d	0.0002160 mg/kg-d	26,544,000	(Tryphonas <i>et al.</i> , 1986).
Humoral immunity	Circulating IgM response (SRBC antigen; hemolysis)	Monkey (females)	Aroclor 1254	Route: oral (juice) Duration: 5/w; 27-28 mo	0.2000 mg/kg-d	0.0002160 mg/kg-d	26,544,000	(Tryphonas <i>et al.</i> , 1986)

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Category	Endpoint	Species (sex)	Test article	Dosing parameters	Animal NOAEL dose	HED, dioxin-equivalents	MOE	Reference
Organ weight, histopath	Organ weight, histopath	Monkey (females)	Aroclor 1254	Route: oral (juice) Duration: 5/w; 12-13 mo	0.2000 mg/kg-d	0.0002160 mg/kg-d	26,544,000	(Tryphonas <i>et al.</i> , 1986)
Organ weight, histopath	Organ weight, histopath	Monkey (females)	Aroclor 1254	Route: oral (juice) Duration: 5/w; 27-28 mo	0.2000 mg/kg-d	0.0002160 mg/kg-d	26,544,000	(Tryphonas <i>et al.</i> , 1986)
Resistance	Malaria	Mouse (males)	Aroclor 1016	Route: oral (diet) Duration: 1/d; 6w	1.0000 mg/kg-d	0.0000002 mg/kg-d	27,000	(Loose <i>et al.</i> , 1977; Loose <i>et al.</i> , 1978a; Loose <i>et al.</i> , 1979)
Other	Liver lesions with MLV infection	Mouse (males)	Aroclor 1221	Route: oral (diet) Duration: 1/d; 6 mo	70.0000 mg/kg-d	0.0000056 mg/kg-d	688,000	(Koller, 1977)
Cell mediated immunity	Cytotoxic T Lymphocyte assay P815	Mouse (not specified)	Aroclor 1016	Route: oral (diet) Duration: 1/d; 40 w	30.0000 mg/kg-d	0.0000066 mg/kg-d	811,000	(Silkworth and Loose, 1981)
Cell mediated immunity	Graft-vs-host response	Mouse (males)	Aroclor 1016	Route: oral (diet) Duration: 1/d; Spleen index assessed 9d after inoculation	30.0000 mg/kg-d	0.0000066 mg/kg-d	811,000	(Silkworth and Loose, 1979a)
Cell mediated immunity	Graft-vs-host response	Mouse (not specified)	Aroclor 1016	Route: oral (diet) Duration: 1/d; 37 w	30.0000 mg/kg-d	0.0000066 mg/kg-d	811,000	(Silkworth and Loose, 1981)
Cell mediated immunity	Lymphocyte blastogenesis, T cell mitogen(s) PHA	Mouse (males)	Aroclor 1016	Route: oral (diet) Duration: 1/d; 41w	30.0000 mg/kg-d	0.0000066 mg/kg-d	811,000	(Silkworth and Loose, 1979a)
Cell mediated immunity	Lymphocyte blastogenesis, T cell mitogen(s) PHA	Mouse (male)	Aroclor 1016	Route: oral (diet) Duration: 1/d; 40 w	30.0000 mg/kg-d	0.0000066 mg/kg-d	811,000	(Silkworth and Loose, 1981)

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Category	Endpoint	Species (sex)	Test article	Dosing parameters	Animal NOAEL dose	HED, dioxin-equivalents	MOE	Reference
Cell mediated immunity	Mixed Lymphocyte reaction Splenocytes	Mouse (male)	Aroclor 1016	Route: oral (diet) Duration: 1/d; 40 w	30.0000 mg/kg-d	0.0000066 mg/kg-d	811,000	(Silkworth and Loose, 1981)
Organ weight, histopath	Organ weight, histopath	Mouse (males)	Aroclor 1016	Route: oral (diet) Duration: 1/d; 41w	30.0000 mg/kg-d	0.0000066 mg/kg-d	811,000	(Silkworth and Loose, 1979a)
Nonspecific immunity	Killing assay	Mouse (males)	Aroclor 1242	Route: oral (diet) Duration: 1/d; 18w	0.9000 mg/kg-d	0.0000124 mg/kg-d	1,526,000	(Loose <i>et al.</i> , 1981)
Resistance	EL-4 tumor cell injection	Mouse (males)	Aroclor 1242	Route: oral (diet) Duration: 1/d; 18w	0.9000 mg/kg-d	0.0000124 mg/kg-d	1,526,000	(Loose <i>et al.</i> , 1981)
Resistance	Malaria	Mouse (males)	Aroclor 1242	Route: oral (diet) Duration: 1/d; 6w	1.0000 mg/kg-d	0.0000138 mg/kg-d	1,696,000	(Loose <i>et al.</i> , 1977; Loose <i>et al.</i> , 1978a; Loose <i>et al.</i> , 1979)
Other	Reactive oxygen species (spleen, thymus supernatant)	Mouse (both)	PCB126	Route: oral (gavage) Duration: 1/d; Up to 7 d	0.0005 mg/kg-d	0.0000500 mg/kg-d	6,144,000	(Du <i>et al.</i> , 2019)
Cell mediated immunity	Lymphocyte blastogenesis, T cell mitogen(s) ConA	Mouse (both (pooled))	Mixture (Aroclor1 ³⁰² 42:Aroclor1 254 2:1)	Route: oral (diet) Duration: 1/d; to 22 w of age	4.7000 mg/kg-d	0.0000523 mg/kg-d	6,430,000	(Segre <i>et al.</i> , 2002)
Humoral immunity	Circulating IgG response (KLH antigen; ELISA)	Mouse (both (pooled))	Mixture (Aroclor124 2:Aroclor12 54 2:1)	Route: oral (diet) Duration: 1/d; to 22 w of age	4.7000 mg/kg-d	0.0000523 mg/kg-d	6,430,000	(Segre <i>et al.</i> , 2002)

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Category	Endpoint	Species (sex)	Test article	Dosing parameters	Animal NOAEL dose	HED, dioxin-equivalents	MOE	Reference
Humoral immunity	PFC response (T-independent; DNP antigen)	Mouse (females exposed before mating; both sexes evaluated)	Kanechlor 500	Route: oral (gavage) Duration: 2/w; 3 w exposure to dams prior to mating, cross-over with controls during lactation to generate pre+postnatal, prenatal, or postnatal exposure grps. Offspring sac 5 d after DNP immunization	14.0000 mg/kg-d	0.0000896 mg/kg-d	11,011,000	(Takagi <i>et al.</i> , 1987)
Humoral immunity	PFC response (T-independent; DNP antigen)	Mouse (females exposed before mating; both sexes evaluated)	Kanechlor 500	Route: oral (gavage) Duration: 2/w; 3 w exposure to dams prior to mating, cross-over with controls during lactation to generate pre+postnatal, prenatal, or postnatal exposure grps. Bacterial alpha-amylase (BaA) or DNP-KLH injections to mice generated BaA or DNP-KLH primed spleen cells for	14.0000 mg/kg-d	0.0000896 mg/kg-d	11,011,000	(Takagi <i>et al.</i> , 1987)
Resistance	Ectromelia virus	Mouse (males)	Kanechlor 500	Route: diet Duration: daily; 21 d	18.0000 mg/kg-d	0.0001152 mg/kg-d	14,157,000	(Imanishi <i>et al.</i> , 1980)
Resistance	Herpes simplex virus [HSV]	Mouse (males)	Kanechlor 500	Route: diet Duration: daily; 21 d	18.0000 mg/kg-d	0.0001152 mg/kg-d	14,157,000	(Imanishi <i>et al.</i> , 1980)
Resistance	Listeria, HSV-2	Mouse (not specified)	Aroclor 1254	Route: oral (gavage) Duration: 1/d; 14 days prior to i.v. injection of microorganism	30.0000 mg/kg-d	0.0001740 mg/kg-d	21,382,000	(Bradley and Morahan, 1982)

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Category	Endpoint	Species (sex)	Test article	Dosing parameters	Animal NOAEL dose	HED, dioxin-equivalents	MOE	Reference
Cell mediated immunity	Delayed-type hypersensitivity response	Mouse (both)	Aroclor 1254	Route: oral (diet) Duration: 1/d; to 8 w of age	41.7000 mg/kg-d	0.0002419 mg/kg-d	29,722,000	(Talcott and Koller, 1983)
Humoral immunity	Circulating antibody response (BSA antigen; ELISA)	Mouse (both)	Aroclor 1254	Route: oral (diet) Duration: 1/d; to 8 w of age	41.7000 mg/kg-d	0.0002419 mg/kg-d	29,722,000	(Talcott and Koller, 1983)
Nonspecific immunity	Phagocytotic activity	Mouse (both)	Aroclor 1254	Route: oral (diet) Duration: 1/d; to 8 w of age	41.7000 mg/kg-d	0.0002419 mg/kg-d	29,722,000	(Talcott and Koller, 1983)
Nonspecific immunity	Killing assay	Mouse (males)	Aroclor 1242	Route: oral (diet) Duration: 1/d; 18w	18.0000 mg/kg-d	0.0002484 mg/kg-d	30,525,000	(Loose <i>et al.</i> , 1981)
Nonspecific immunity	Killing assay	Mouse (males)	Aroclor 1242	Route: oral (diet) Duration: 1/d; 18w	18.0000 mg/kg-d	0.0002484 mg/kg-d	30,525,000	(Loose <i>et al.</i> , 1981)
Nonspecific immunity	Phagocytotic activity	Mouse (males)	Aroclor 1242	Route: oral (diet) Duration: 1/d; 18w	18.0000 mg/kg-d	0.0002484 mg/kg-d	30,525,000	(Loose <i>et al.</i> , 1981)
Other	Oxygen consumption (macrophages, PMNs, dead yeast)	Mouse (males)	Aroclor 1242	Route: oral (diet) Duration: 1/d; 18w	18.0000 mg/kg-d	0.0002484 mg/kg-d	30,525,000	(Loose <i>et al.</i> , 1981)
Resistance	L1210 tumor cells	Mouse (males)	Aroclor 1242	Route: oral (diet) Duration: 1/d; 18w	18.0000 mg/kg-d	0.0002484 mg/kg-d	30,525,000	(Loose <i>et al.</i> , 1981)
Resistance	mKSA tumor cell injection	Mouse (males)	Aroclor 1242	Route: oral (diet) Duration: 1/d; 18w	18.0000 mg/kg-d	0.0002484 mg/kg-d	30,525,000	(Loose <i>et al.</i> , 1981)

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Category	Endpoint	Species (sex)	Test article	Dosing parameters	Animal NOAEL dose	HED, dioxin-equivalents	MOE	Reference
Resistance	P388 tumor cells	Mouse (males)	Aroclor 1242	Route: oral (diet) Duration: 1/d; 18w	18.0000 mg/kg-d	0.0002484 mg/kg-d	30,525,000	(Loose <i>et al.</i> , 1981)
Humoral immunity	Circulating IgG, IgM, IgA (radial immunodiffusion, RID)	Mouse (males)	Aroclor 1242	Route: oral (diet) Duration: 1/d; sac 4-7 days following SRBC injection	30.0000 mg/kg-d	0.0004140 mg/kg-d	50,876,000	(Loose <i>et al.</i> , 1977; Loose <i>et al.</i> , 1978a)
Other	Serum interferon inducibility	Mouse (males)	Kanechlor 500	Route: diet Duration: daily; 21 d	66.0000 mg/kg-d	0.0004224 mg/kg-d	51,908,000	(Imanishi <i>et al.</i> , 1980)
Organ weight, histopath	Organ weight, histopath	Mouse (female)	Aroclor 1248	Route: oral (diet) Duration: 1/d; 5 w	200.0000 mg/kg-d	0.0056000 mg/kg-d	688,172,000	(Thomas and Hinsdill, 1978)
Other	Potentialiation of endometriosis lesion	Mouse (females)	PCB126	Route: oral (gavage) Duration: 3 w btwn doses for 5 total doses; 16 w	1.0000 mg/kg-d	0.1000000 mg/kg-d	12,288,786,000	(Johnson <i>et al.</i> , 1997)
Nonspecific immunity	NK cell activity	Rat (males)	Aroclor 1254	Route: oral (gavage) Duration: 1/d; 15w	1.0000 mg/kg-d	0.0000324 mg/kg-d	3,982,000	(Smialowicz <i>et al.</i> , 1989)
Other	2-year Chronic bioassay, immune organ effects	Rat (females)	PCB118	Route: oral (gavage) Duration: 5d/w; 105 w	1.0000 mg/kg-d	0.0000540 mg/kg-d	6,636,000	(NTP, 2010)
Other	2-year Chronic bioassay, immune organ effects	Rat (females)	PCB126	Route: oral (gavage) Duration: 5d/w; 105 w	0.0001 mg/kg-d	0.0000540 mg/kg-d	6,636,000	(NTP, 2006c)

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Category	Endpoint	Species (sex)	Test article	Dosing parameters	Animal NOAEL dose	HED, dioxin-equivalents	MOE	Reference
Humoral immunity	Circulating IgG response (KLH antigen; 1° response; ELISA)	Rat (male)	Aroclor 1254	Route: oral (diet) Duration: 1/d; 9 w blood collected up to 15d after initial KLH injection	4.3000 mg/kg-d	0.0001393 mg/kg-d	17,121,000	(Koller <i>et al.</i> , 1983b)
Cell mediated immunity	Lymphocyte blastogenesis, T cell mitogen(s) PHA	Rat (males)	Aroclor 1254	Route: oral (gavage) Duration: 1/d; 15w	10.0000 mg/kg-d	0.0003240 mg/kg-d	39,816,000	(Smialowicz <i>et al.</i> , 1989)
Humoral immunity	Lymphocyte blastogenesis, B,T cell mitogen(s) PWM	Rat (males)	Aroclor 1254	Route: oral (diet) Duration: 1/d; 7 d	22.0000 mg/kg-d	0.0007128 mg/kg-d	87,594,000	(Bonnyns and Bastomsky, 1976)
Cell mediated immunity	Cytotoxic T Lymphocyte assay	Rat (males)	Aroclor 1254	Route: oral (gavage) Duration: 1/d; 15w	25.0000 mg/kg-d	0.0008100 mg/kg-d	99,539,000	(Smialowicz <i>et al.</i> , 1989)
Cell mediated immunity	Lymphocyte blastogenesis, T cell mitogen(s) ConA	Rat (males)	Aroclor 1254	Route: oral (gavage) Duration: 1/d; 15w	25.0000 mg/kg-d	0.0008100 mg/kg-d	99,539,000	(Smialowicz <i>et al.</i> , 1989)
Cell mediated immunity	Mixed Lymphocyte reaction	Rat (males)	Aroclor 1254	Route: oral (gavage) Duration: 1/d; 15w	25.0000 mg/kg-d	0.0008100 mg/kg-d	99,539,000	(Smialowicz <i>et al.</i> , 1989)
Humoral immunity	Lymphocyte blastogenesis, B cell mitogen(s) STM	Rat (males)	Aroclor 1254	Route: oral (gavage) Duration: 1/d; 15w	25.0000 mg/kg-d	0.0008100 mg/kg-d	99,539,000	(Smialowicz <i>et al.</i> , 1989)
Humoral immunity	Lymphocyte blastogenesis, B,T cell mitogen(s) PWM	Rat (males)	Aroclor 1254	Route: oral (gavage) Duration: 1/d; 15w	25.0000 mg/kg-d	0.0008100 mg/kg-d	99,539,000	(Smialowicz <i>et al.</i> , 1989)

Category	Endpoint	Species (sex)	Test article	Dosing parameters	Animal NOAEL dose	HED, dioxin-equivalents	MOE	Reference
Humoral immunity	Circulating IgG response (KLH antigen; 2° response; ELISA)	Rat (male)	Aroclor 1254	Route: oral (diet) Duration: 1/d; 9 w blood collected up to 21d after initial KLH injection	43.0000 mg/kg-d	0.0013932 mg/kg-d	171,207,000	(Koller <i>et al.</i> , 1983b)

Table A4-9. MOEs for LOAELs of immunotoxicity in PCB studies of mice, rats and monkeys with 2 or more oral administrations

Category	Endpoint	Species (sex)	Test article	Dosing parameters	Animal LOAEL dose	HED, dioxin-equivalents	MOE	Reference
Circulating factors	↑alpha1-thymosin	Monkey (female)	Aroclor 1254	Route: oral (gelatin capsule) Duration: 1/d; 55 mo	0.0050 mg/kg-d	0.0000054 mg/kg-d	664,000	(Tryphonas <i>et al.</i> , 1991b)
Humoral immunity	Circulating IgG response (SRBC antigen; hemagglutination)	Monkey (female)	Aroclor 1254	Route: oral (gelatin capsule) Duration: 1/d; 23 mo	0.0050 mg/kg-d	0.0000054 mg/kg-d	664,000	(Tryphonas <i>et al.</i> , 1989)
Humoral immunity	Circulating IgM response (SRBC antigen; hemolysis)	Monkey (female)	Aroclor 1254	Route: oral (gelatin capsule) Duration: 1/d; 23 mo	0.0050 mg/kg-d	0.0000054 mg/kg-d	664,000	(Tryphonas <i>et al.</i> , 1989)
Humoral immunity	Circulating IgM response (SRBC antigen; hemolysis)	Monkey (female)	Aroclor 1254	Route: oral (gelatin capsule) Duration: 1/d; 55 mo	0.0050 mg/kg-d	0.0000054 mg/kg-d	664,000	(Tryphonas <i>et al.</i> , 1991a)
Nonspecific immunity	Complement activity	Monkey (female)	Aroclor 1254	Route: oral (gelatin capsule) Duration: 1/d; 55 mo	0.0050 mg/kg-d	0.0000054 mg/kg-d	664,000	(Tryphonas <i>et al.</i> , 1991b)

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Category	Endpoint	Species (sex)	Test article	Dosing parameters	Animal LOAEL dose	HED, dioxin-equivalents	MOE	Reference
Organ weight, histopath	Organ weight, histopath	Monkey (not specified [both])	Aroclor 1248	Route: in utero and nursing Duration: 1/d; 18 months (6 mo before breeding to control males, throughout gestation, and 3 mo after delivery). Infants placed on milk supplement at 4 mo of age	0.1000 mg/kg-d	0.0000072 mg/kg-d	885,000	(Allen and Barsotti, 1976)
Humoral immunity	Circulating IgG response (SRBC antigen; hemagglutination)	Monkey (both)	Mixture (major PCBs in Canadian human milk)	Route: oral (formula) Duration: 1/d; 20 wks exposure, 66 wks total (w/ follow-up)	0.0075 mg/kg-d	0.0000072 mg/kg-d	885,000	(Arnold <i>et al.</i> , 1999)
Humoral immunity	Circulating gamma globulin (electrophoresis)	Monkey (female)	Aroclor 1248	Route: oral (diet) Duration: 1/d; ~16 mo	0.2000 mg/kg-d	0.0000144 mg/kg-d	1,770,000	(Thomas and Hinsdill, 1978)
Humoral immunity	Circulating IgM response (SRBC antigen; hemolysis)	Monkey (female)	Aroclor 1248	Route: oral (diet) Duration: 1/d; ~16 mo	0.2000 mg/kg-d	0.0000144 mg/kg-d	1,770,000	(Thomas and Hinsdill, 1978)
Organ weight, histopath	Organ weight, histopath	Monkey (not specified [both])	Aroclor 1248	Route: in utero and nursing Duration: 1/d; Discontinued treated diet of mothers 1 yr before mating	0.2000 mg/kg-d	0.0000144 mg/kg-d	1,770,000	(Allen <i>et al.</i> , 1980)
Humoral immunity	Circulating IgG response (SRBC antigen; hemagglutination)	Monkey (female)	Aroclor 1254	Route: oral (gelatin capsule) Duration: 1/d; 55 mo	0.0400 mg/kg-d	0.0000432 mg/kg-d	5,309,000	(Tryphonas <i>et al.</i> , 1991a)
Cell subset distributions	Circulating cells	Monkey (female)	Aroclor 1254	Route: oral (gelatin capsule) Duration: 1/d; 23 mo	0.0800 mg/kg-d	0.0000864 mg/kg-d	10,618,000	(Tryphonas <i>et al.</i> , 1989)

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Category	Endpoint	Species (sex)	Test article	Dosing parameters	Animal LOAEL dose	HED, dioxin-equivalents	MOE	Reference
Organ weight, histopath	Organ weight, histopath	Monkey (both (1 mo old at study start))	Aroclor 1248	Route: oral (gavage) Duration: 1/d; 30 days	35.0000 mg/kg-d	0.0025200 mg/kg-d	309,677,000	(Abrahamson and Allen, 1973)
Resistance	Endotoxin	Mouse (male)	Aroclor 1016	Route: oral (diet) Duration: 1/d; 3w	1.0000 mg/kg-d	0.0000002 mg/kg-d	27,000	(Loose <i>et al.</i> , 1977; Loose <i>et al.</i> , 1978a; Loose <i>et al.</i> , 1979)
Other	Liver lesions with MLV infection	Mouse (male)	Aroclor 1254	Route: oral (diet) Duration: 1/d; 6 mo	0.7000 mg/kg-d	0.0000041 mg/kg-d	499,000	(Koller, 1977)
Cell mediated immunity	Lymphocyte blastogenesis, T cell mitogen(s)	Mouse (not specified)	Aroclor 1016	Route: oral (diet) Duration: 1/d; up to 41w	30.0000 mg/kg-d	0.0000066 mg/kg-d	811,000	(Silkworth and Loose, 1979b)
Cell mediated immunity	Mixed Lymphocyte reaction	Mouse (male)	Aroclor 1016	Route: oral (diet) Duration: 1/d; 24 w	30.0000 mg/kg-d	0.0000066 mg/kg-d	811,000	(Silkworth and Loose, 1979b)
Other	Liver lesions with MLV infection	Mouse (male)	Aroclor 1242	Route: oral (diet) Duration: 1/d; 6 mo	0.7000 mg/kg-d	0.0000097 mg/kg-d	1,187,000	(Koller, 1977)
Circulating factors	Serum fibronectin	Mouse (male)	Aroclor 1242	Route: oral (diet) Duration: 1/d; 18w	0.9000 mg/kg-d	0.0000124 mg/kg-d	1,526,000	(Loose <i>et al.</i> , 1981)
Resistance	Endotoxin	Mouse (male)	Aroclor 1242	Route: oral (diet) Duration: 1/d; 3w	1.0000 mg/kg-d	0.0000138 mg/kg-d	1,696,000	(Loose <i>et al.</i> , 1977; Loose <i>et al.</i> , 1978a; Loose <i>et al.</i> , 1979)
Cell subset distributions	Circulating cells	Mouse (both)	PCB126	Route: oral (gavage) Duration: 1/d; Up to 7 d	0.0005 mg/kg-d	0.0000500 mg/kg-d	6,144,000	(Du <i>et al.</i> , 2019)

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Category	Endpoint	Species (sex)	Test article	Dosing parameters	Animal LOAEL dose	HED, dioxin-equivalents	MOE	Reference
Circulating factors	Circulating cytokines	Mouse (both)	PCB126	Route: oral (gavage) Duration: 1/d; Up to 7 d	0.0005 mg/kg-d	0.0000500 mg/kg-d	6,144,000	(Du <i>et al.</i> , 2019)
Organ weight, histopath	Organ weight, histopath	Mouse (both)	PCB126	Route: oral (gavage) Duration: 1/d; Up to 7 d	0.0005 mg/kg-d	0.0000500 mg/kg-d	6,144,000	(Du <i>et al.</i> , 2019)
Other	Reactive oxygen species generation in plated spleen cells	Mouse (both)	PCB126	Route: oral (gavage) Duration: 1/d; Up to 7 d	0.0005 mg/kg-d	0.0000500 mg/kg-d	6,144,000	(Du <i>et al.</i> , 2019)
Cell mediated immunity	Lymphocyte blastogenesis, T cell mitogen(s) ConA	Mouse (both (pooled))	Mixture (Aroclor124 2:Aroclor1254 2:1)	Route: oral (diet) Duration: 1/d; to 22 w of age	4.7000 mg/kg-d	0.0000523 mg/kg-d	6,430,000	(Segre <i>et al.</i> , 2002)
Organ weight, histopath	Organ weight, histopath	Mouse (both (pooled))	Mixture (Aroclor124 2:Aroclor1254 2:1)	Route: oral (diet) Duration: 1/d; to 22 w of age	4.7000 mg/kg-d	0.0000523 mg/kg-d	6,430,000	(Segre <i>et al.</i> , 2002)
Resistance	Ectromelia virus	Mouse (male)	Kanechlor 500	Route: diet Duration: daily; 21 d	33.0000 mg/kg-d	0.0002112 mg/kg-d	25,954,000	(Imanishi <i>et al.</i> , 1980)
Resistance	Herpes simplex virus [HSV]	Mouse (male)	Kanechlor 500	Route: diet Duration: daily; 21 d	33.0000 mg/kg-d	0.0002112 mg/kg-d	25,954,000	(Imanishi <i>et al.</i> , 1980)
Nonspecific immunity	Killing assay	Mouse (male)	Aroclor 1242	Route: oral (diet) Duration: 1/d; 18w	18.0000 mg/kg-d	0.0002484 mg/kg-d	30,525,000	(Loose <i>et al.</i> , 1981)
Resistance	EL-4 tumor cell injection	Mouse (male)	Aroclor 1242	Route: oral (diet) Duration: 1/d; 18w	18.0000 mg/kg-d	0.0002484 mg/kg-d	30,525,000	(Loose <i>et al.</i> , 1981)

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Category	Endpoint	Species (sex)	Test article	Dosing parameters	Animal LOAEL dose	HED, dioxin-equivalents	MOE	Reference
Humoral immunity	Circulating IgG, IgM, IgA (radial immunodiffusion, RID)	Mouse (male)	Aroclor 1242	Route: oral (diet) Duration: 1/d; sac 4-7 days following SRBC injection	30.0000 mg/kg-d	0.0004140 mg/kg-d	50,876,000	(Loose <i>et al.</i> , 1977; Loose <i>et al.</i> , 1978a)
Humoral immunity	Circulating IgG, IgM, IgA (radial immunodiffusion, RID)	Mouse (male)	Aroclor 1242	Route: oral (diet) Duration: 1/d; sac 4-11 days following SRBC injection	30.0000 mg/kg-d	0.0004140 mg/kg-d	50,876,000	(Loose <i>et al.</i> , 1977; Loose <i>et al.</i> , 1978a)
Humoral immunity	PFC response (T-dependent; SRBC antigen)	Mouse (male)	Aroclor 1242	Route: oral (diet) Duration: 1/d; sac 4-7 days following SRBC injection	30.0000 mg/kg-d	0.0004140 mg/kg-d	50,876,000	(Loose <i>et al.</i> , 1977; Loose <i>et al.</i> , 1978a)
Humoral immunity	PFC response (T-dependent; SRBC antigen)	Mouse (male)	Aroclor 1242	Route: oral (diet) Duration: 1/d; sac 4-7 days following SRBC injection	30.0000 mg/kg-d	0.0004140 mg/kg-d	50,876,000	(Loose <i>et al.</i> , 1977; Loose <i>et al.</i> , 1978a)
Humoral immunity	PFC response (T-dependent; SRBC antigen)	Mouse (male)	Aroclor 1242	Route: oral (diet) Duration: 1/d; sac 4-11 days following SRBC injection	30.0000 mg/kg-d	0.0004140 mg/kg-d	50,876,000	(Loose <i>et al.</i> , 1977; Loose <i>et al.</i> , 1978a)
Resistance	Endotoxin	Mouse (male)	Aroclor 1242	Route: oral (diet) Duration: 1/d; 6w	30.0000 mg/kg-d	0.0004140 mg/kg-d	50,876,000	(Loose <i>et al.</i> , 1978b)
Resistance	Malaria	Mouse (male)	Aroclor 1242	Route: oral (diet) Duration: 1/d; 6w	30.0000 mg/kg-d	0.0004140 mg/kg-d	50,876,000	(Loose <i>et al.</i> , 1978a; Loose <i>et al.</i> , 1978b)
Other	Reactive oxygen species (spleen, thymus supernatant)	Mouse (both)	PCB126	Route: oral (gavage) Duration: 1/d; Up to 7 d	0.0050 mg/kg-d	0.0005000 mg/kg-d	61,444,000	(Du <i>et al.</i> , 2019)

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Category	Endpoint	Species (sex)	Test article	Dosing parameters	Animal LOAEL dose	HED, dioxin-equivalents	MOE	Reference
Resistance	Endotoxin	Mouse (female)	Aroclor 1248	Route: oral (diet) Duration: 1/d; 5 w	20.0000 mg/kg-d	0.0005600 mg/kg-d	68,817,000	(Thomas and Hinsdill, 1978)
Resistance	Salmonella typhimurium	Mouse (female)	Aroclor 1248	Route: oral (diet) Duration: 1/d; 5 w	200.0000 mg/kg-d	0.0056000 mg/kg-d	688,172,000	(Thomas and Hinsdill, 1978)
Humoral immunity	Circulating antibody response (BSA antigen; ELISA)	Rat (male)	Aroclor 1254	Route: oral (gavage) Duration: 2/w; 14 w blood collected on d96	0.0300 mg/kg-d	0.0000010 mg/kg-d	119,000	(Koller <i>et al.</i> , 1983a)
Circulating factors	Corticosterone	Rat (male)	Aroclor 1254	Route: oral (gavage) Duration: 1/d; 15w	0.1000 mg/kg-d	0.0000032 mg/kg-d	398,000	(Miller <i>et al.</i> , 1993)
Other	2-year Chronic bioassay, immune organ effects	Rat (female)	PCB126	Route: oral (gavage) Duration: 5d/w; 105 w	0.0002 mg/kg-d	0.0000945 mg/kg-d	11,613,000	(NTP, 2006c)
Humoral immunity	Circulating IgG response (KLH antigen; 2° response; ELISA)	Rat (male)	Aroclor 1254	Route: oral (diet) Duration: 1/d; 10 w	4.3000 mg/kg-d	0.0001393 mg/kg-d	17,121,000	(Exon <i>et al.</i> , 1985)
Nonspecific immunity	NK cell activity	Rat (male)	Aroclor 1254	Route: oral (diet) Duration: 1/d; 10 w	4.3000 mg/kg-d	0.0001393 mg/kg-d	17,121,000	(Exon <i>et al.</i> , 1985)
Nonspecific immunity	NK cell activity	Rat (male)	Aroclor 1254	Route: oral (diet) Duration: feed; 10 w	4.3000 mg/kg-d	0.0001393 mg/kg-d	17,121,000	(Talcott <i>et al.</i> , 1985)
Other	IL-2 release, splenocytes, bkg levels following KLH injections	Rat (male)	Aroclor 1254	Route: oral (diet) Duration: 1/d; 10 w	4.3000 mg/kg-d	0.0001393 mg/kg-d	17,121,000	(Exon <i>et al.</i> , 1985)

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Category	Endpoint	Species (sex)	Test article	Dosing parameters	Animal LOAEL dose	HED, dioxin-equivalents	MOE	Reference
Other	2-year Chronic bioassay, immune organ effects	Rat (female)	PCB118	Route: oral (gavage) Duration: 5d/w; 105 w	4.6000 mg/kg-d	0.0002484 mg/kg-d	30,525,000	(NTP, 2010)
Nonspecific immunity	NK cell activity	Rat (male)	Aroclor 1254	Route: oral (gavage) Duration: 1/d; 15w	10.0000 mg/kg-d	0.0003240 mg/kg-d	39,816,000	(Smialowicz <i>et al.</i> , 1989)
Cell mediated immunity	Lymphocyte blastogenesis, T cell mitogen(s) PHA	Rat (male)	Aroclor 1254	Route: oral (diet) Duration: 1/d; 7 d	22.0000 mg/kg-d	0.0007128 mg/kg-d	87,594,000	(Bonnyns and Bastomsky, 1976)
Cell mediated immunity	Lymphocyte blastogenesis, T cell mitogen(s) PHA	Rat (male)	Aroclor 1254	Route: oral (gavage) Duration: 1/d; 15w	25.0000 mg/kg-d	0.0008100 mg/kg-d	99,539,000	(Smialowicz <i>et al.</i> , 1989)
Humoral immunity	Circulating IgG response (KLH antigen; 1° response; ELISA)	Rat (male)	Aroclor 1254	Route: oral (diet) Duration: 1/d; 9 w blood collected up to 15d after initial KLH injection	43.0000 mg/kg-d	0.0013932 mg/kg-d	171,207,000	(Koller <i>et al.</i> , 1983b)

Non dioxin-like PCBs

Although it has been well established that the mode of action for PCB-induced immunotoxicity is via the AhR (see Section II.C), a few animal studies evaluated the immunotoxic effects of PCBs that are not associated with AhR activation (i.e., the non-dioxin like PCBs or the non-DL PCBs). For completeness, a similar MOE assessment was performed to assess the relationship between the reasonable upper bound estimate of human exposure and the levels of exposure tested in laboratory animals. My analysis used a similar approach to calculate human equivalent doses from laboratory animal studies that I then compared to an estimate of the upper bound of human exposure.

Immune-related tests of exclusively non-dioxin like congeners using an oral route of administration and 2 or more administrations were seldom performed. The NOAEL and LOAEL dataset was therefore much smaller than that involving dioxin-like PCBs. No monkey NOAEL or LOAEL values were available for non-dioxin like congeners; studies of rats identified 3 NOAELs and 1 LOAEL; and studies of mice identified 1 NOAEL and 1 LOAEL.

Human equivalent doses were derived from respective animal NOAEL and LOAEL values using applicable animal to human body size correction factors. Comparisons to potential human exposure were performed using total intake of all PCB congeners (i.e., the sum of all congeners ingested daily), which simplified the calculations and likely over-estimated potential exposures. Reasonable upper bound estimates of total PCB intake were calculated using (1) mean total PCB tissue concentrations together with 95th percentiles for consumption levels by Spokane River consumers (meaning that 95% of consumption was at or less than the value used) and (2) the upper 95th percentiles of total PCB tissue concentrations with the mean levels of Spokane River fish consumption. The resulting values were 1,115 and 417 ng/day, respectively (see Section II.A). I used the more conservative (i.e., higher) value of 1,115 ng/day as the predicted human intake of total PCBs in this MOE analysis.³¹

To calculate MOEs for non-dioxin like PCBs specific to immunotoxic endpoints, I used the following equation:

Equation 2. Margin of Exposure calculation for non-dioxin like PCBs

$$MOE(non - DL PCBs) = \frac{HED \left(\frac{mg}{kg \text{ bw}} \right)}{[Total PCBs] \left(\frac{ng}{day} \right) * \left(\frac{1 \text{ mg}}{10^6 ng} \right) * \left(\frac{1}{80kg} \right)}$$

where HED was the human equivalent dose for immune-related NOAELs and LOAELs for non-dioxin like PCBs from laboratory animal studies and [total PCBs] was the predicted human intake of total PCBs, converted to mg/kg-d assuming an average human bodyweight of 80 kg (US EPA, 2011a).

Equation 2 was applied to each of the mouse and rat studies that used an oral route of administration and 2 or more administrations of non-dioxin like PCBs. Resulting MOEs for each NOAEL and LOAEL are included in Table A4-10 and Table A4-11, respectively. The limited amount of non-dioxin like tests and their associated

³¹ Use of total PCB intake rather than congener-specific intake is a conservative approach because it overestimates individual congener intake and many animal studies studied effects of single PCB congeners.

MOEs are evident in their presentation in Figure A4-3. For consistency, the same layout used for the dioxin-like PCBs was used in this figure even though most categories had no data.

Many of the MOEs were associated with NOAELs that corresponded to the highest administered dose and suggest the absence of immunotoxic effects upon exposure to non-dioxin like PCBs. The remaining LOAEL-based MOEs were greater than 100,000, which means that the estimated reasonable upper bound estimate of exposure in humans is more than 100,000-times above the levels tested in animal studies and thus do not present a risk of immunotoxicity with human consumption.

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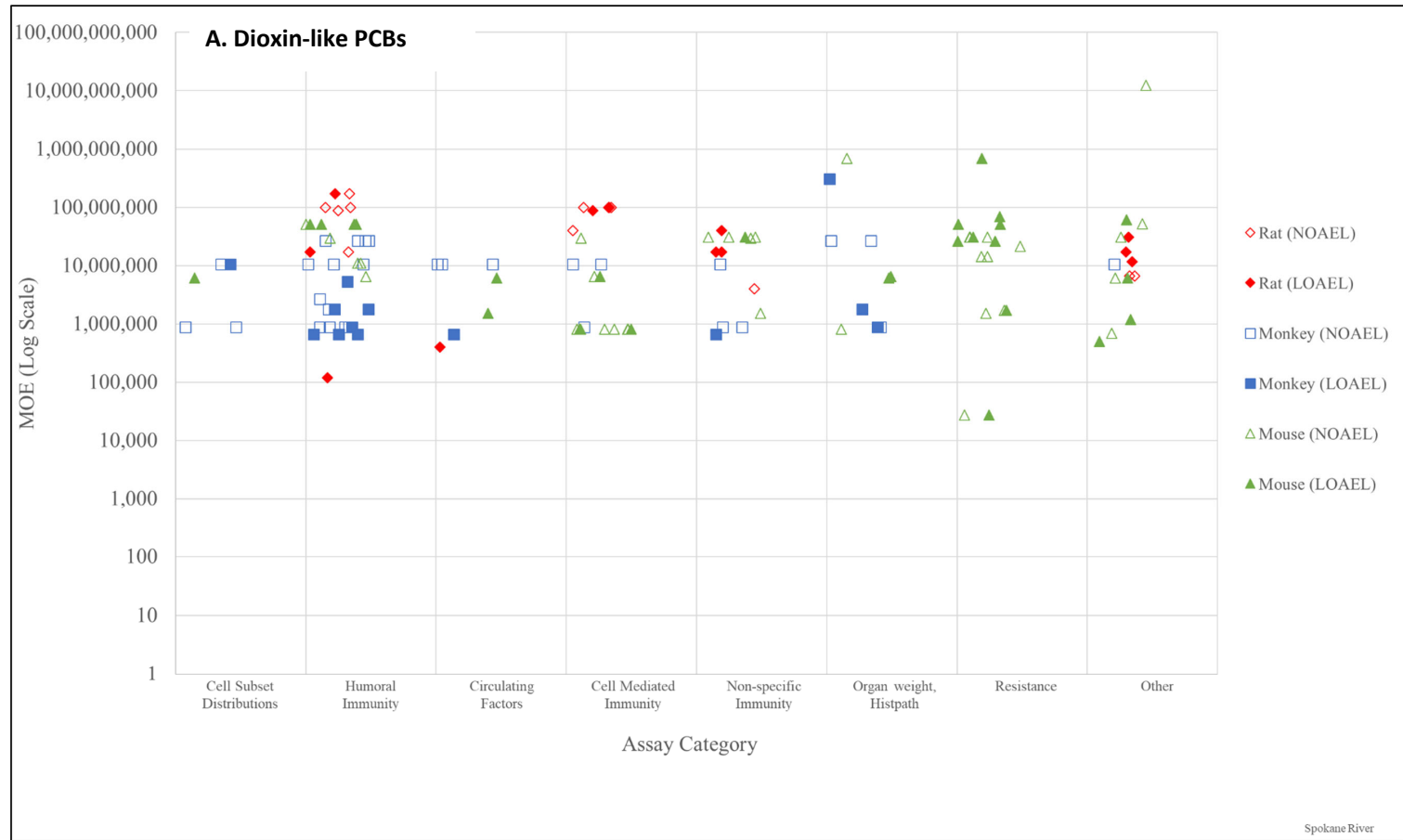


Figure A4-3. Estimated MOEs the Spokane River fish consumption associated with immune-related NOAELs and LOAELs from laboratory animal studies of dioxin-like (Panel A) and non-dioxin like (Panel B) PCB congeners

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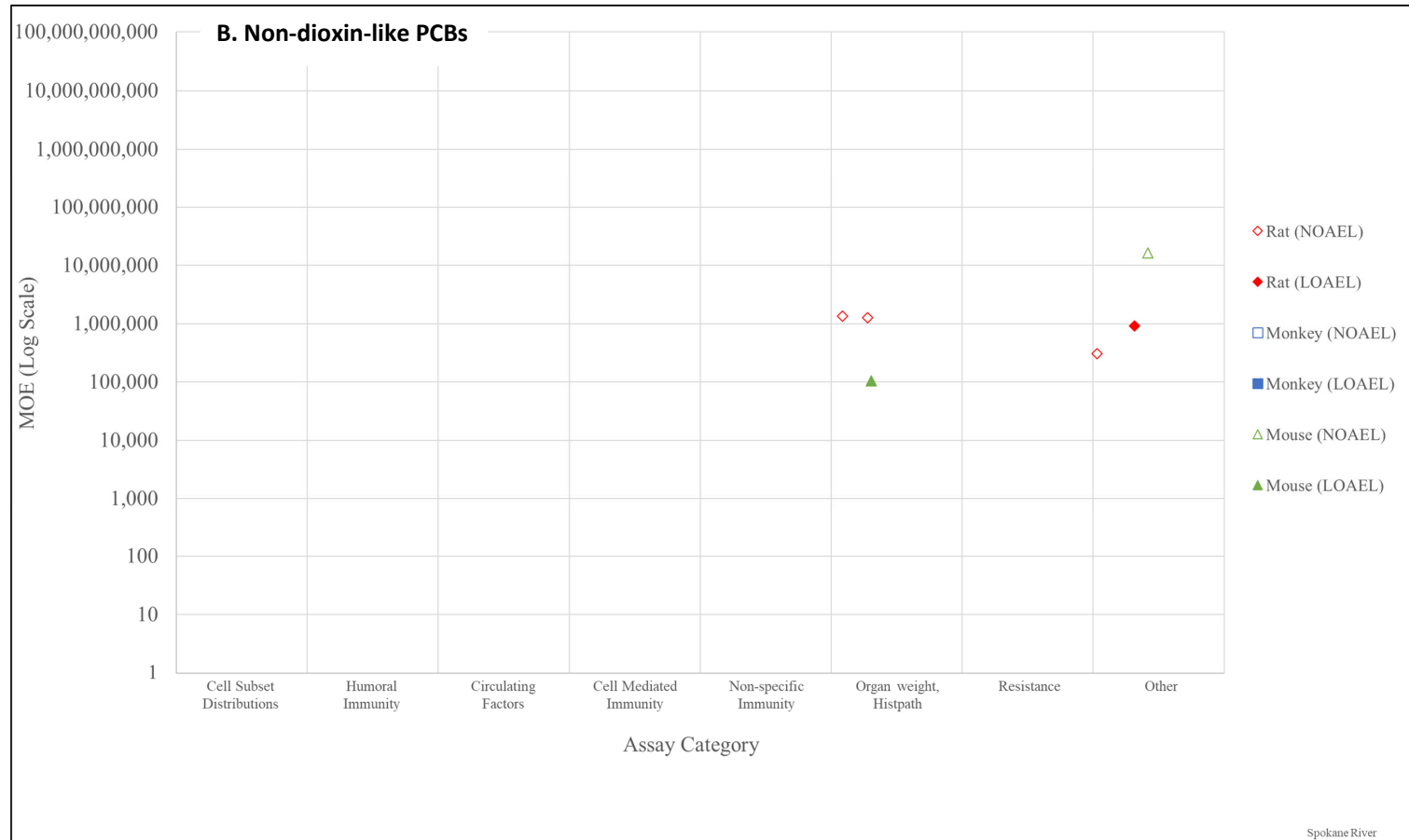


Figure A4-3. Estimated MOEs the Spokane River fish consumption associated with immune-related NOAELs and LOAELs from laboratory animal studies of dioxin-like (Panel A) and non-dioxin like (Panel B) PCB congeners, continued

Table A4-10. MOEs for NOAELs of immunotoxicity in non-dioxin like PCB studies of mice, rats and monkeys with 2 or more oral administrations

Category	Endpoint	Species (sex)	Test article	Dosing parameters	Animal NOAEL dose	MOE	Reference
Other	Potential of endometriosis lesion	Mouse (female)	PCB153	Route: oral (gavage) Duration: 3 w btwn doses for 5 total doses; 16 w	30 mg/kg-d	16,200,000	(Johnson <i>et al.</i> , 1997)
Other	2-year Chronic bioassay, immune organ effects	Rat (female)	PCB153	Route: oral (gavage) Duration: 5d/w; 105 w	1 mg/kg-d	303,000	(NTP, 2006a)
Organ weight, histopath	Organ weight, histopath	Rat (male)	PCB128	Route: oral (diet) Duration: 1/d; 13 w	4.2 mg/kg-d	1,275,000	(Lecavalier <i>et al.</i> , 1997)
Organ weight, histopath	Organ weight, histopath	Rat (female)	PCB128	Route: oral (diet) Duration: 1/d; 13 w	4.4 mg/kg-d	1,335,000	(Lecavalier <i>et al.</i> , 1997)

Table A4-11. MOEs for LOAELs of immunotoxicity in non-dioxin like PCB studies of mice, rats and monkeys with 2 or more oral administrations

Category	Endpoint	Species (sex)	Test article	Dosing parameters	Animal LOAEL dose	MOE	Reference
Organ weight, histopath	Organ weight, histopath	Mouse (female)	PCB153	Route: oral (diet) Duration: 1/d; 28d	0.1950 mg/kg-d	105,000	(Maranghi <i>et al.</i> , 2013)
Other	2-year Chronic bioassay, immune organ effects	Rat (female)	PCB153	Route: oral (gavage) Duration: 5d/w; 105 w	3.0000 mg/kg-d	910,000	(NTP, 2006a)

IX. Appendix 5: Neurodevelopmental toxicology of PCBs

A. Introduction to risk assessment for adverse neurodevelopmental outcomes

Numerous investigations since the 1970s have assessed the potential for PCBs to cause neurodevelopmental effects in animals, including effects on cognition, behavior, motor skills, and sensory systems. Neurodevelopmental research on PCBs in animals began to receive attention in the late 1970s following a human PCB/dibenzofuran intoxication event in Japan in 1968 in which contaminated rice bran oil was ingested by a large population (Chou *et al.*, 1979). Along with the skin, eyes, liver, and endocrine organs, the nervous system was one of the primary affected organs/tissues in exposed individuals. Neurologic symptoms included headaches, numbness, hypoesthesia and neuralgia. Although PCBs from a leaking heat-exchange were the source of the contamination, chemical analysis of the contaminated cooking oil revealed extraordinarily high levels of chlorinated dibenzofurans, which, as discussed previously, are potent agonists of the human AhR. The contribution of PCB congeners to the total 'TEQ' exposure, using current TEF values, represented approximately 0.2% of the total TEQ. The vast majority of the TEQ was from pentachlorodibenzofuran (Todaka *et al.*, 2016). Nevertheless, this tragic event triggered awareness of the potential neurotoxic effects of dioxin-like compounds, and led to numerous neurodevelopmental studies on PCBs conducted in several species (including nonhuman primates, NHPs) using a wide range of methodologies and PCB congeners/mixtures.

A toxicological assessment of the animal literature was performed to assess whether consumption of PCBs in fish from the Spokane River is likely to cause neurodevelopmental effects. The approach used is detailed below and followed a standard risk assessment framework, including hazard identification, dose-response assessment, exposure assessment, and risk characterization (Faustman and Omenn, 2013).

B. Literature search approach

To identify neurodevelopmental studies relevant for my risk assessment, I conducted a review of the animal literature. My emphasis was on primary literature (*i.e.*, no review articles, meta-analyses, *etc.*); however, I did review reports from authoritative bodies such as Agency for Toxic Substances and Disease Registry (ATSDR), United States Environmental Protection Agency (US EPA), *etc.* Combinations of search terms³² were used to query PubMed and Google Scholar for studies assessing the potential cognitive, behavioral, motor, and sensory effects of PCBs in animals. Since both dioxin-like (DL) and non-dioxin like (NDL) PCBs have been associated with neurodevelopmental effects in animals, studies were included that evaluated both types of congeners and congener mixtures.

³² Search terms included: ("polychlorinated biphenyls" OR PCBs) AND (neurodev* OR neurotox* OR neurochem* OR neurobeh* OR behavior* OR brain OR activ* OR learn* OR memory OR social) AND (pups OR pup OR offspring OR infant* OR gestation* OR weaning OR lactat* OR dam OR dams OR mother*) AND (animal* OR rat OR rats OR mouse OR mice OR monkey* OR hamster* OR pig OR pigs OR piglet* OR sheep OR cow OR cows); ("polychlorinated biphenyls" OR PCBs) AND (motor OR locomotor OR hyperactivity OR coordination OR anxiety OR emotion OR social OR auditor* OR smell OR sweet OR coch* OR sensory) AND (pups OR pup OR offspring OR infant* OR gestation* OR weaning OR lactat* OR dam OR dams OR mother*) AND (animal* OR rat OR rats OR mouse OR mice OR monkey* OR hamster* OR pig OR pigs OR piglet* OR sheep OR cow OR cows OR rabbit OR rabbits).

A total of approximately 450 studies were identified based on these search terms. Of these studies, 87 passed the criteria outlined in Table A5-1 and were considered in my toxicological assessment.

Table A5-1. Number of animal studies in database passing sequential criteria for evaluation of neurodevelopmental effects following exposure to PCBs

Criterion	No. of Studies
Neurodevelopmental studies*	~450
<i>In vivo</i> studies [†]	106
Oral administration	98
Repeated dosing (<i>i.e.</i> , no bolus dosing)	93
Dosing during pre-gestation, gestation, or lactation	90
Relevant endpoints assessed [‡]	87
Conducted in relevant animal models [§]	87

* Based on queries of PubMed and Google Scholar using search terms listed in main text. Studies with only abstracts available were not included in my assessment. This literature search was updated on September 18, 2019.

[†] Excluding *in vitro* or mechanistic studies and studies administering PCB metabolites

[‡] Cognitive, behavioral, motor, and sensory effects

[§] Monkeys, rats, or mice

The 87 studies are provided in Attachment 4. Of these, 10 were conducted in NHPs and 77 were conducted in rats or mice. To support the hazard and dose-response assessments (Sections C and D of this appendix), relevant information for each study including species (and strain), exposure route, exposure frequency and duration, administered dose(s),³³ PCB congener(s) or mixture, age at assessment, neurobehavioral test(s), and endpoint(s) were compiled. For each neurobehavioral test within a study, a no-observed-adverse-effect-level (NOAEL) and/or lowest-observed-adverse-effect-level (LOAEL) was identified based on reported statistical significance: an administered dose was identified as a LOAEL if an effect was statistically significant at a p-value < 0.05 when compared to controls. If no statistical evaluation was conducted by the authors, NOAELs and/or LOAELs were not assigned. The NOAELs and LOAELs selected in this manner are assessed for toxicological relevance in the dose-response section of this report. The NOAELs and LOAELs were adjusted to a human-equivalent dose (HED) according to body size scaling factors per (US EPA, 2011d) guidance regarding comparison of laboratory animal doses to potential human exposure.

C. Hazard identification

In this section I describe the primary neurodevelopmental effects investigated in the PCB animal study literature, and the neurodevelopmental tests used to assess these endpoints. In addition, I describe the proposed modes of action for neurodevelopmental effects of PCBs based on studies carried out *in vitro*.

1. Overview of neurodevelopmental effects and tests

³³ When a study reported the administered dose in terms of quantity in feed, I derived a body weight dose (mg PCB/kg body weight per day) based on food consumption and body weight data provided in the study or default values from the literature. In addition, if dosing was intermittent instead of daily, I derived a daily dose based on exposure duration and frequency.

Neurodevelopmental effects associated with PCB exposure in animals have been shown for cognitive, behavioral (*e.g.*, response inhibition, anxiety, activity), motor, and sensory domains. These effects have been reported for commercial mixtures (*e.g.*, Aroclors), defined experimental mixtures with environmental relevance, and single congeners. Table A5-2 lists the numbers of studies by species assessed in the filtered literature for categories of neurodevelopmental effects and PCB congener or mixture.

Cognitive and behavioral endpoints have received the most attention, while motor activity and sensory endpoints are less represented. While cognitive and behavioral endpoints are generally assessed via separate tests in rodents, tests in NHPs may include measurements for either endpoint. No studies evaluated sensory endpoints in NHPs. Studies in NHPs administered an Aroclor mixture (1248 or 1016) or a mixture of 15 congeners representative of human breast milk³⁴ (Rice, 1997). Rodent studies dosing with single congeners predominantly used PCB126 (a DL congener) or PCB153 (an NDL congener); a number of different commercial and environmental mixtures were administered to mice and rats.

Cognitive effects include both learning and memory. Learning is operationally defined as how well or quickly a reinforced behavior is acquired, or how well a subject adjusts a response strategy to changes in the presentation of the test or reward. Learning in animals is evaluated using various tests, including discrimination testing (*i.e.*, rewarding selection of stimuli such as colors, shapes, or spatial cues in NHPs or spatial forms in maze testing in rodents) and schedule-controlled behavior tests (*i.e.*, fixed interval (FI) or fixed ratio (FR) tests in which rewards are tied to either a specific number of required responses or to a temporal relationship between responses and reward).

³⁴ This mixture of 15 congeners was formulated to represent 80% of the congeners present in breast milk from Canadian women, in a proportional mixture.

Table A5-2. Number of studies represented by selected experimental parameters from the neurodevelopmental literature on PCB effects in animals

Parameter	NHP	Mouse	Rat
Effect			
Cognition/behavior*	8*	NA	NA
Cognition	NA	9	34
Behavior	NA	3	3
Motor activity	3	12	18
Sensory	0	4	26
PCB congener or mixture			
Single congener	0	4	24
Aroclors [†]	6	5	32
Environmental mixture [‡]	4 [§]	6	7
PCB structural class			
Dioxin-like (DL)	0	3	14
Non-dioxin like (NDL)	0	5	15
DL + NDL	10	7	37

*Neurodevelopmental tests in NHPs include measurements for both cognitive and behavioral endpoints.

†Other commercial mixtures included (*e.g.*, Kanechlors, Clophens, *etc.*).

‡Mixture formulated to include congeners present in environmentally relevant matrices (*e.g.*, human breast milk, contaminated fish, *etc.*).

§ The same mixture of 15 congeners (representative of PCBs in human breast milk) was used in the NHP studies.

NA - not applicable.

Memory is generally assessed as either working (short-term) memory or reference (long-term) memory. For example, in delayed spatial alternation (DSA) tests, NHPs are taught to alternate between left and right stimuli, and working memory is then tested by adding in a delay, typically 5 to 40 seconds, after reinforcement prior to presentation of the next trial. A subject must recall which stimulus produced the reward on their last selection in order to make the next correct selection. In rodents, working and reference memory may be assessed using the radial arm maze (which consists of a central location and several radial pathways that may be baited with food) and reference memory via the Morris water maze (which assesses whether the subject recalls the location of the hidden platform by measuring the time required for a subject to escape from a pool of water via finding and then recalling the location of hidden platforms).

Behavioral effects such as response inhibition, or impulsivity, have been tested using a type of schedule-control testing termed differential reinforcement of low rate (DRL). In DRL tests, a delay is implemented between reinforcement and the next trial, during which any response goes unrewarded and resets the delay counter. Emotional behavior, particularly anxiety, has been assessed in rodents using a variety of approaches (Sousa *et al.*, 2006). Two of the most common tests are the light/dark box and the elevated plus maze, which rely on rodents' natural aversion to open, bright spaces (Sousa *et al.*, 2006; Walf and Frye, 2007). Anxiety may also be assessed in rodents via novelty/sociability tests, in which a rodent may explore either a space including a familiarized starting compartment, a compartment with a confined rodent with no prior socialization history, and an empty compartment. This test may be extended by adding a rodent into the

empty compartment in a follow-up test to assess novelty preference between the first rodent and the second rodent. These tests, however, may be confounded by generalized activity changes. Depression is sometimes tested in rodents via a tail suspension test, in which latency to go limp is measured.

Motor function is measured as either overall activity levels or as functional observational batteries (FOBs) at a young age. General activity levels are generally assessed via open field or activity chamber tests in which a subject is placed in a rectangular area that is sectioned into grids. The subject's movement across the grids is then measured, either via manual counting or through automated observation. FOBs include straightforward tests of fine and gross motor control, including tests of grip strength, climbing, swimming, and postural stability (often via rotarod, in which a rat's ability to stay on a rotating cylinder is assessed). Another test is the water escape pole climbing (WESPOC) test, which assesses visuomotor coordination by require a rat to find and climb a pole to escape a pool of water.

Sensory functioning includes visual, auditory, and somatic acuity and may be assessed in NHPs by determining whether they can perceive differently spaced black and white bands, tonal frequencies, or vibrations on their fingers (Burbacher and Grant, 2000). Visual and auditory acuity in rodents can be assessed via cliff avoidance tests, in which a young rat must perceive the presence of a steep drop, or acoustic startle, in which the presence of a reflex response to a tone is assessed.

For all of these endpoints, especially cognitive and behavioral effects, it is important to note that neurodevelopmental tests may be more or less sensitive to subtle behavioral changes depending on the difficulty of the task presented. In terms of relevance of animal models to human neurodevelopment, important advantages to using NHPs for neurodevelopmental testing are apparent when compared to rodent models (Slikker *et al.*, 2018). First, in rodents, higher cognitive functions are either lacking or technically difficult to evaluate. Second, rodents significantly differ from humans and NHPs in terms of their anatomy, fine motor skills, and dominant sensory modality. Third, development of the rodent central nervous system (CNS) differs temporally from humans and NHPs (*e.g.*, birth of granule cells in cerebellar cortex, myelinogenesis). While acknowledging these differences, I consider studies in both NHPs and rodents in my assessment.

2. Modes of action for neurodevelopmental effects

Unlike the cancer and immunotoxicity endpoints discussed in this report, the mechanistic basis for neurodevelopmental effects of PCBs is not well-established. Furthermore, both DL- and NDL-PCBs have been associated with neurodevelopmental effects in animals. Multiple modes of action for neurodevelopmental effects have been proposed and reported *in vitro*, including enhanced ryanodine receptor activity, decreased dopamine content, perturbations in calcium homeostasis, activation of aryl hydrocarbon receptor (AhR), protein kinase C (PKC) translocation, and perturbation of G-protein-coupled receptor (GPCR) signaling, among others (Choi *et al.*, 2016; Kodavanti, 2005; Mariussen and Fonnum, 2006; Pessah *et al.*, 2010; Pradeep *et al.*, 2019). For example, some PCB congeners (*e.g.*, PCB95) have been shown to activate the ryanodine receptor, a Ca^{2+} ion channel expressed by neurons in the mammalian brain, leading to increased intracellular Ca^{2+} levels (Pessah *et al.*, 2010). Changes in intracellular Ca^{2+} are a key mode of action by which neuronal activity regulates neurodevelopmental processes that in turn determine synaptic connectivity (Bal-Price *et al.*, 2017).

Because the mechanistic basis for neurodevelopmental effects of PCBs is an active area of research and may involve multiple pathways, I did not use an Adverse Outcomes Pathway (AOP) approach for my assessment of neurodevelopmental effects and instead relied on standard toxicological approaches. As described in my report, AOP analyses use mode of action or mechanistic data (e.g., activation of AhR) to develop points of departure or benchmark doses for chemicals, but this approach requires that the modes of action be well understood.

D. Dose-response assessment

In this section, I assess the relationship between the dose of PCBs and incidence of neurodevelopmental effects in animals. I separate my evaluation by species, focusing on NHPs and then rodents. Section E of this appendix describe estimates for the maximum potential exposure rates of PCBs to consumers of fish caught from the Spokane River, and Section F describes calculation of Margins of Exposure (MOEs) comparing points of departure (NOAELs or LOAELs) identified in neurodevelopmental tests in animals to the estimated PCB consumption rates for congeners or mixtures from fish. MOEs are calculated for all of the neurodevelopmental tests performed in studies that passed the described literature search screening criteria (Section B). However, this dose-response assessment discussion is focused on studies that assessed exposure of NHPs or rodents to low, environmentally-relevant dose levels. Results from these studies are described in detail below, as well as results from studies that use the same neurodevelopmental tests to evaluate offspring of animals exposed to much higher doses. Because variability in study design and methodological issues impact reliability and replicability of study results, these issues are also discussed below.

1. Reliability and consistency of experimental parameters across literature

For empirical findings to be reliable, they must be verified by measurement or observation across multiple studies; *i.e.*, the literature findings must be repeated, replicated, and reproduced (Plesser, 2017).³⁵ This is especially critical for assessment of neurobehavior, where individual differences are common, leading to increased within-group variability (Kafkafi *et al.*, 2018). In addition, tests that measure behavioral constructs such as “anxiety” or “behavioral despair” are not well validated, particularly since it is not well understood what these tests actually measure. For example, variability in results from the tail suspension and Morris water maze tests in mice may be explained by behaviors other than behavioral despair or memory.

Key experimental parameters vary widely across the neurodevelopmental literature on PCBs and impact the ability to identify a reliable point of departure for evaluating dose-response. A point of departure is defined

³⁵ From Plesser, 2017:

Repeatability (Same team, same experimental setup): The measurement can be obtained with stated precision by the same team using the same measurement procedure, the same measuring system, under the same operating conditions, in the same location on multiple trials.

Replicability (Different team, same experimental setup): The measurement can be obtained with stated precision by a different team using the same measurement procedure, the same measuring system, under the same operating conditions, in the same or a different location on multiple trials.

Reproducibility (Different team, different experimental setup): The measurement can be obtained with stated precision by a different team, a different measuring system, in a different location on multiple trials.

as a dose (*e.g.*, NOAEL or LOAEL) that is used as the starting point for subsequent dose-response extrapolations and analyses. Some of these issues are described below:

- *PCB congener and/or mixture*: Of the 209 PCB congeners, only a small fraction has been toxicologically assessed individually, with most individual congener studies focusing on PCB126 because of its dioxin-like activity. While a number of different commercial PCB mixtures have been assessed (*e.g.*, Aroclors, Kanechlors, Clophens, *etc.*), the formulation of these mixtures has varied over time. In addition, environmentally relevant PCB mixtures (*e.g.*, formulations mimicking congeners found in human breast milk, contaminated fish, *etc.*) can vary across studies. Only recently has there been a push to standardize experimental mixtures across studies, for example using a mixture of six environmental “indicator” PCBs (EFSA, 2005). Overall, single congener studies of NDLS and NDL-only mixtures are under-represented in the literature, despite their relevance to environmental exposures and potential neurodevelopmental effects.
- *Impurities in PCB formulations*: Studies (especially older studies) have not sufficiently documented impurities in PCB formulations (*e.g.*, contamination due to DL-PCBs, polychlorobenzodioxins (PCDDs), polychlorodibenzofurans (PCDFs), *etc.*). Even at >99% purity, minor contamination by DL-PCBs can confound toxicological assessments due to the high potency of DL-PCBs (ATSDR, 2000; Viluksela *et al.*, 2012).
- *Number of doses and sample size*: Many studies only include only one dose level (*i.e.*, one treatment group), which is below the minimum number of doses recommended in guidelines for animal toxicity testing by authoritative bodies (*e.g.*, EPA, OECD). In particular, only two of the eight NHP cohorts studied included more than one dose group. Studies with only one dose do not allow for an assessment of dose-response under the conditions of the study. Regardless, given the prevalence of studies with only one treatment group across the literature, I considered these studies in my evaluation. Furthermore, the sample sizes used in NHP studies were relatively small, with treatment groups ranging in size from 3 to 8 animals and control groups ranging from 4 to 7 animals.
- *Exposure period and frequency*: The developmental period when animals were dosed was variable across studies. Studies dosed animals prior to gestation, during gestation, during lactation, or a combination of the prior. Animals were also dosed during different windows within gestation and/or lactation (*e.g.*, gestation day (GD) 10-16, GD 7-21). Exposure frequencies were daily, intermittent, or bolus (note, in my selection of an appropriate point of departure, I did not consider studies using only bolus dosing since the relevant exposure scenario (*i.e.*, fish consumption) involves repeated exposures).
- *Age at assessment*: Neurodevelopmental assessments were conducted in offspring at varying life stages following developmental exposure, including weanling, pubescent, young adult and adult life stages. A neurodevelopmental test may have been used to assess a different life stage in another study, such that findings from the two studies are not directly comparable.
- *Neurodevelopmental test and test measurement*: An array of neurodevelopmental tests was implemented, with variations in objective and implementation. In NHPs, few tests were repeated across multiple studies. In addition, different measurements were often collected from the same

tests. For example, measurements collected in schedule of reinforcement testing included some combination of interresponse time (IRT), number of IRTs less than a certain time interval, pause time, index of curvature (IOC), number of reinforced responses, and number of nonreinforced responses, etc., with different statistical approaches applied in the analyses of the data.

- *Lab or investigator:* Many of the neurodevelopmental studies assessing PCBs in animals were conducted by the same research groups, especially those studies that administered environmentally relevant doses. Consequently, intralaboratory bias could impact the study results. For example, the majority of studies in NHPs were conducted between 1970 and the 1990s by two groups: the University of Wisconsin and Health Canada (these studies are summarized in Figure A5-1). More recent studies in mice using NDL mixtures in the ng/kg-d range were carried out by the same group of French and Spanish investigators based at the Université de Lorraine (Dridi *et al.*, 2014; Elnar *et al.*, 2016; Elnar *et al.*, 2012; Karkaba *et al.*, 2017); the findings from these studies have not been replicated elsewhere.

Figure A5-1 illustrates the variability in experimental parameters in studies of neurodevelopmental effects of PCBs in NHPs.

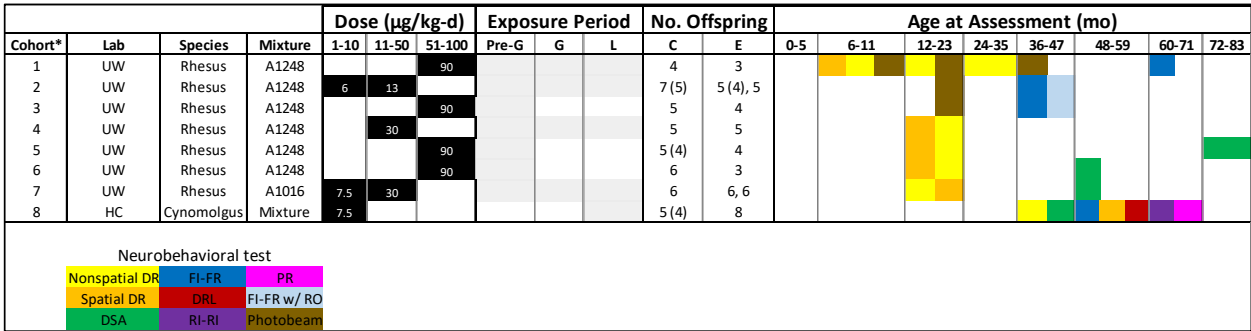


Figure A5-1. Overview of experimental designs of neurobehavioral studies in nonhuman primates assessing effects of PCBs.

*Cohort number refers to the following studies: 1 – Bowman and Heironimus (1981); Bowman *et al.* (1978); Bowman *et al.* (1981); Mele *et al.* (1986); 2 – Bowman *et al.* (1981); Mele *et al.* (1986); 3 – Mele *et al.* (1986) 4 – Schantz *et al.* (1989); 5 – Levin *et al.* (1988); Schantz *et al.* (1989); 6 – Levin *et al.* (1988); Schantz *et al.* (1989); 7 – Levin *et al.* (1988); Schantz *et al.* (1989); 8 – Rice (1997, 1998); Rice and Hayward (1997, 1998). Note that cohorts 3, 5, and 6 are so-called post-exposure cohorts whose mothers' exposure to PCBs ceased roughly 1, 1.5, or 2.6 years prior to breeding. Note additionally that the University of Wisconsin cohorts are not always clearly defined across studies and I used my best judgement to identify re-used groups. ; A, Aroclor; C, control group; DR, discrimination learning; DRL, differential reinforcement of low rate; DSA, delayed spatial alternation; E, exposure group; FI, fixed-interval; FR, fixed ratio; G, gestation; HC, Health Canada; L, lactation; PR, progressive ratio; RI, random interval; RO, response inhibition; UWisc – University of Wisconsin. ()-indicates that a smaller number of animals was used in follow-up testing; if multiple dose groups were tested, the dose group number of animals are listed from smallest to greatest dose and are separated by an ' '.

2. Nonhuman primate studies

Based on the results of my literature search, I identified 10 studies that assessed neurodevelopmental effects of PCBs in NHPs. These 10 studies assessed cohorts administered PCB mixtures at doses ranging from 7.5 to

90 µg/kg-d. No studies assessed NHPs at higher doses. The University of Wisconsin (UWisc) conducted testing in the late 1970s through the 1980s in seven cohorts of male and female rhesus macaque (*Macaca mulatta*) offspring—some cohorts shared the same mothers— and the Toxicology Research Division of Health Canada (HC) conducted testing in a single cohort of male cynomolgus (*Macaca fascicularis*) monkeys from 1997 to 1999. The UWisc cohorts were exposed to PCBs via mothers who were dosed prior to gestation through weaning at 4 months, while the HC cohort was directly dosed during the first 20 weeks after birth. The cohorts and their exposures are summarized in Figure A5-1 and Table A5-3. Note that both laboratories reused NHP cohorts in multiple studies. UWisc dosed with Aroclors, either 1248 or 1016, while HC dosed with a mixture formulated to mimic the distribution of congeners identified in the breast milk of Canadian women. No single-congener studies were conducted in NHPs. Neurodevelopmental effects reported in the NHP studies are summarized in Table A5-4.

Overall, interpretation of test results does not support significant effects on learning or memory in either the HC or UWisc cohorts of NHPs. A test for response inhibition identified effects in the HC cohort exposed to 7.5 µg/kg-d of the breast milk mixture; these effects were inconsistently seen in comparable measurements on the same cohort or on cohorts dosed with 6 or 90 µg/kg-d Aroclor 1248 by UWisc. Effects on motor activity were reported in two cohorts exposed to 90 µg/kg-d Aroclor 1248 by UWisc, one perinatally and one via rebred mothers with no concurrent exposure. No effects were found in a third cohort exposed to 6.3 µg/kg-d Aroclor 1248 by UWisc (this cohort included a 13 µg/kg-d dose group that was not tested for locomotor activity). At the 90 µg/kg-d dose, both high and low levels of activity were found compared to controls depending on age. I discuss these results in more detail below

Table A5-3. Nonhuman primate studies assessing neurodevelopmental effects of prenatal/lactational exposure to PCBs

Cohort	Study	Species	Congener or Mixture	Doses (µg/kg-d)*	Period of Administration
1 (UWisc)	Bowman and Heironimus (1981); Bowman <i>et al.</i> (1978); Bowman <i>et al.</i> (1981); Mele <i>et al.</i> (1986);	Rhesus	Aroclor 1248	90	~22 months, ending at weaning
2 (UWisc)	Bowman <i>et al.</i> (1981); Mele <i>et al.</i> (1986)	Rhesus	Aroclor 1248	6.3, 13	16 – 25 months, ending at weaning
3 (UWisc)	Bowman <i>et al.</i> (1981); Mele <i>et al.</i> (1986)	Rhesus	Aroclor 1248	90	~22 months, ending ~20 months prior to conception
4 (UWisc)	Schantz <i>et al.</i> (1989)	Rhesus	Aroclor 1248	13	1 year prior to breeding through weaning at 4 months of age
5 (UWisc)	Levin <i>et al.</i> (1988); Schantz <i>et al.</i> (1989)	Rhesus	Aroclor 1248	90	~18 months, ending 12 months prior to conception
6 (UWisc)	Levin <i>et al.</i> (1988); Schantz <i>et al.</i> (1989)	Rhesus	Aroclor 1248	80	~18 months, ending 32 months prior to conception
7 (UWisc)	Levin <i>et al.</i> (1988); Schantz <i>et al.</i> (1989)	Rhesus	Aroclor 1016	7.6, 30	7 months prior to breeding through weaning at 4 months of age
8 (HC)	Rice (1997, 1998); Rice and Hayward (1997, 1999b)	Cynomolgus	Mixture of 15 NDL-PCBs*	7.5	0-20 wks starting at birth

* If study reported dose relative to body weight, that is reported here. If the dose was reported relative to feed, then dose was derived relative to body weight. Doses shown exclude controls (i.e., 0 µg/kg-d).

† Experimental mixture formulated to represent 80% of the congeners present in breast milk from Canadian women in a proportional mixture. The congeners included: PCB52, PCB66, PCB74, PCB105, PCB118, PCB138, PCB153, PCB156, PCB157, PCB180, PCB183, PCB187, PCB189, PCB194, and PCB203.

Table A5-4. Neurodevelopmental effects associated with prenatal/lactational exposure to PCBs in nonhuman primate studies

Cohort (Lab, Congener or mixture, species)	Study	Cognitive (i.e., learning and memory) or Behavioral (i.e., response inhibition)* (µg/kg-d)	Motor function (i.e., general activity) (µg/kg-d)
1 (UWisc, A1248, Rhesus)	Bowman <i>et al.</i> (1978)	Neurological battery (6-24mo): NOAEL: None LOAEL: 90	Photobeam (6, 12mo): NOAEL: None LOAEL: 90
	Bowman <i>et al.</i> (1981)	NA	Photobeam (6, 12mo): NOAEL: None LOAEL: 90

Cohort (Lab, Congener or mixture, species)	Study	Cognitive (i.e., learning and memory) or Behavioral (i.e., response inhibition)* (µg/kg-d)	Motor function (i.e., general activity) (µg/kg-d)
	Bowman and Heironimus (1981)	NA	Photobeam (44 mo): NOAEL: None LOAEL: 90
	Mele <i>et al.</i> (1986)	Fixed interval (60 mo): NOAEL: 90 LOAEL: None	NA
2 (UWisc, A1248, Rhesus)	Bowman <i>et al.</i> (1981)	NA	Photobeam (12 mo): NOAEL: 13 LOAEL: None
	Mele <i>et al.</i> (1986)	Fixed interval (40 mo): NOAEL: None LOAEL: 6.3 (Note that the high dose group in this cohort was not given this test.) Fixed interval w/ reinforcement omission (40 mo): NOAEL: 6.3 (Note that the high dose group in this cohort was not given this test.) LOAEL: None	NA
3 (UWisc, A1248, Rhesus)	Bowman <i>et al.</i> (1981)	NA	Photobeam (6, 12 mo): NOAEL: None LOAEL: 90
	Mele <i>et al.</i> (1986)	Fixed interval (40 mo): NOAEL: None LOAEL: 90 Fixed interval w/ reinforcement omission (40 mo): NOAEL: None LOAEL: 90	NA
4 (UWisc, A1248, Rhesus)	Schantz <i>et al.</i> (1989)	Discrimination reversal (14 mo): NOAEL: 13 LOAEL: None	NA
5 (UWisc, A1248, Rhesus)	Levin <i>et al.</i> (1988)	Delayed spatial alternation (6 y): NOAEL: None LOAEL: 90	NA
	Schantz <i>et al.</i> (1989)	Discrimination reversal (14 mo): NOAEL: 90 LOAEL: None	NA

Cohort (Lab, Congener or mixture, species)	Study	Cognitive (i.e., learning and memory) or Behavioral (i.e., response inhibition)* (µg/kg-d)	Motor function (i.e., general activity) (µg/kg-d)
6 (UWisc, A1248, Rhesus)	Levin <i>et al.</i> (1988)	Delayed spatial alternation (4 y): NOAEL: None LOAEL: 80	NA
	Schantz <i>et al.</i> (1989)	Discrimination reversal (14 mo): NOAEL: 80 LOAEL: None	NA
7 (UWisc, A1016, Rhesus)	Levin <i>et al.</i> (1988)	Delayed spatial alternation (4 y): NOAEL: 30 LOAEL: None	NA
	Schantz <i>et al.</i> (1989)	Discrimination reversal (14 mo): NOAEL: 7.6 LOAEL: 30	NA
8 (HC, Mixture of 15 NDL-PCBs [†] , Cynomolgus)	Rice and Hayward (1997)	Discrimination reversal, nonspatial (3 y): NOAEL: None LOAEL: 7.5 Delayed spatial alternation (3 y): NOAEL: None LOAEL: 7.5	NA
	Rice (1997)	Fixed ratio (4 y): NOAEL: None LOAEL: 7.5 Fixed interval (4 y): NOAEL: None LOAEL: 7.5	NA
	Rice (1998)	Spatial discrimination reversal (4.5 y): NOAEL: 7.5 LOAEL: None Differential reinforcement of low rate (4.5y): NOAEL: None LOAEL: 7.5	NA

Cohort (Lab, Congener or mixture, species)	Study	Cognitive (i.e., learning and memory) or Behavioral (i.e., response inhibition)* (µg/kg-d)	Motor function (i.e., general activity) (µg/kg-d)
	(Rice and Hayward, 1999b)	Concurrent random interval – random interval (5 y): NOAEL: 7.5 LOAEL: None Progressive ratio task (5.5 y) NOAEL: None LOAEL: 7.5	NA

* To be conservative, LOAELs reported in this table are based on the presence of at least one measurement noted as statistically significant for a test. These measurements are assessed in the text below for toxicological relevance.

† A number of the tests conducted on NHPs include measurements that may be relevant to either cognitive or behavioral endpoints, and so are summarized together in this table.

‡ Experimental mixture formulated to represent 80% of the congeners present in breast milk from Canadian women in a proportional mixture. The congeners included: PCB52, PCB66, PCB74, PCB105, PCB118, PCB138, PCB153, PCB156, PCB157, PCB180, PCB183, PCB187, PCB189, PCB194, and PCB203.

NA - Not applicable

Learning and memory

The literature provides minimal to no evidence for significant effects on learning and memory in NHPs as a result of PCB exposure. Findings from tests of learning are as follows:

- **Progressive ratio:** The HC cohort, with exposure to 7.5 µg/kg-d of a PCB mixture, was tested on this task at 5.5 years of age. Combining data across all sessions of this task, treated NHPs responded more frequently, and persisted in the task longer, than controls (Rice and Hayward, 1999b). However, these differences were driven by differences in early sessions and disappeared by the latter half of testing, indicating no clear learning deficit. No progressive ratio testing was conducted on UWisc cohorts.
- **Fixed-ratio:** No major effects were noted on the HC cohort at 4 years of age, though on an FR-10 schedule (*i.e.*, reinforcement is delivered after every 10 correct responses), treated NHPs had greater pause times than controls (Rice, 1997). No fixed ratio testing was conducted on UWisc cohorts.
- **Concurrent random interval – random interval:** No differences were identified in the HC cohort at 5 years of age (Rice and Hayward, 1999b). No concurrent random interval – random interval testing was conducted on UWisc cohorts.
- **Fixed interval:** No significant effects on learning were identified in the HC cohort at 4.5 years of age or on UWisc cohorts at approximately 3.3 or 5 years of age exposed to 6.3 or 90 µg/kg-d Aroclor 1248 (Cohorts 2 and 3) (Mele *et al.*, 1986; Rice, 1997). A slight, but statistically significant difference in the index of curvature was identified in Cohort 2 (perinatal exposure to 6.3 µg/kg-d Aroclor 1248) at 300 and 600 second intervals and in Cohort 3 (90 µg/kg-d Aroclor 1248, dosing ending ~20 months prior to conception) at 600 second intervals. This measurement indicates that responding occurred

slightly earlier in the interval for treated animals versus controls but is difficult to interpret as a significant effect, especially when no changes were identified in Cohort 1 (perinatal exposure to 90 µg/kg-d Aroclor 1248).

- **Differential rate of reinforcement (DRL):** The HC cohort learned this schedule less well than controls when tested at roughly 5.5 years of age, however the gap in performance diminished by the end of the schedule (Rice, 1998). This test primarily assesses response inhibition and is discussed in more detail below. No DRL testing was conducted on UWisc cohorts.
- **Discrimination reversal:** No major effects were noted in the HC cohort at 3 and 4.5 years of age, including spatial, shape, and color tasks, although controls showed greater latency in responding than treated animals on the shape task, and treated animals showed greater variability in their latency on form and color tasks (Rice, 1998; Rice and Hayward, 1997). UWisc conducted discrimination reversal testing on Cohorts 4, 5, 6, and 7 at 14 months of age and primarily found no effects, with some inconsistent differences from controls and no clear dose-response (Schantz *et al.*, 1989).). In Cohort 7, dose groups exposed to 7.6 or 30 µg/kg-d Aroclor 1016 both performed better than controls on a shape discrimination reversal task, while the 30 µg/kg-d dose group performed worse than controls on a spatial task. Cohort 6, whose mothers' exposure to 80 µg/kg-d Aroclor 1248 ended roughly 32 months prior to conception, performed better than controls in the same shape task as Cohort 7 did, and showed no difference compared to controls on other tasks. Cohort 4, with a perinatal 13 µg/kg-d Aroclor 1248 exposure, showed no differences compared to controls on any discrimination reversal task. Cohort 1, with perinatal exposure to 90 µg/kg-d Aroclor 1248, was also assessed via discrimination reversal testing; however, these results are not interpreted here as no raw data was presented, and the study's analysis depended upon regressions that were not uniformly calculated (Bowman *et al.*, 1978).
- **Delayed spatial alternation:** When tested at 3 years of age, differences between treated and control NHPs in the HC cohort were identified inconsistently at short delays (differenced noted at 0.1, 0.5, and 3 seconds, but not at 1 second), however no differences were identified at 5-30 second delays or under a variable delay structure (Rice and Hayward, 1997). UWisc conducted delayed spatial alternation testing on Cohorts 5, 6 and 7 (Levin *et al.*, 1988). Cohort 7 (perinatal exposure to Aroclor 1016 at 7.6 or 29.7 µg/kg-d) showed no effects. Cohorts 5 and 7 (maternal exposure to 90 µg/kg-d Aroclor 1248 ending 1 year prior to conception or 80 or µg/kg-d Aroclor 1248 ending 32 months prior to conception) showed differences in accuracy (averaging 56.5% correct to the controls' 65.8% correct), however the analysis combined the two cohorts despite their different dosing gap, different effective dose, and different test times (6 vs. 4 years old). Without the raw data available to distinguish between these factors, it is difficult to assess these results.

Response inhibition

Testing focusing specifically on behavioral, non-learning, or memory endpoints was limited in NHPs, with a single DRL test conducted on only one cohort at one dose level. No other tests were conducted that explicitly assessed response inhibition with as sensitive a method, however, certain measures in schedule of reinforcement tests may be comparable, including inter-response time (IRT), pause time, and burst response

(i.e., IRT<5 seconds), among others. Findings from tests and measurements pertaining to response inhibition are discussed below:

- **DRL:** When tested at 5.5 years of age, the HC cohort (Cohort 8) made more incorrect responses than controls, learned the schedule more slowly than controls, and had shorter mean IRTs when a 30-second delay was imposed (Rice, 1998). No differences compared to controls were noted at lower delays, although only the 30-second delay was tested for more than several sessions. UWisc did not conduct DRL testing.
- **Progressive ratio:** No difference in mean IRT was noted in the HC cohort at roughly 5.5 years of age (Rice and Hayward, 1999b). No progressive ratio testing was conducted on UWisc cohorts.
- **Concurrent random interval – random interval:** No differences were identified in the HC cohort at 5 years of age (Rice and Hayward, 1999b). No concurrent random interval – random interval testing was conducted on UWisc cohorts.
- **Fixed interval:** The HC cohort had a shorter mean IRT and more responses within 5 second intervals when tested at 4 years of age, although this behavior decreased over the course of the test (Rice, 1997). Fixed interval testing was conducted by UWisc on Cohorts 1, 2, and 3. No significant effects in response inhibition were noted in Cohorts 1 and 2 (perinatal exposure to 90 or 6.3 µg/kg-d Aroclor 1248). Cohort 3 (maternal exposure to 90 µg/kg-d Aroclor 1248 roughly 1.5 years prior to conception) did show a statistically greater number of responses per second when a reinforcement-omission component was included. No other effects were noted in the reinforcement omission test, and no effects were found in Cohort 2 in the same test (Mele *et al.*, 1986).
- **Fixed ratio:** No differences in pause time, IRT, or IRTs within 5 seconds were noted in the HC cohort tested at 4 years of age, with the exception of greater pause times compared to controls on the FR-10 schedule (Rice, 1997). No fixed ratio testing was conducted on UWisc cohorts.

Motor activity

Tests of motor activity conducted by UWisc report yielded mixed results (Bowman and Heironimus, 1981; Bowman *et al.*, 1981). NHPs perinatally exposed to 6.3 or 13 µg/kg-d Aroclor 1248 showed no difference compared to controls either within or across sessions at 12 months, while NHPs exposed to 90 µg/kg-d became more active relative to controls over the course of sessions at 6 and 12 months, but were less active relative to controls at 44 months. A postexposure group showed greater activity than controls at 12 months, although less so than the 90 µg/kg-d concurrent exposure group at 12 months. No such tests were conducted on the HC cohort.

3. Rodent studies

Based on the results of my literature search, I identified 77 studies conducted in mice or rats as relevant to assessing the potential for neurodevelopmental effects from exposure to PCBs in fish from the Spokane River.

The majority (n=64) of the rodent studies administered PCBs at high doses, *i.e.*, greater than 10 µg/kg-d (Figure A5-2). Of the 13 studies that dosed below this level, only four administered PCBs at doses within ranges that are environmentally relevant based on the MOE analysis, which I summarize in Section F (*i.e.*,

corresponding to doses in the ng/kg-d range). While my assessment of dose-response focuses on the low dose studies, given their relevance to levels of exposure associated with intake of PCBs in Spokane River fish, I also considered studies that evaluated higher doses and compared outcomes reported in those studies to those observed in the lowest dose studies.

The four low dose rodent studies and their dosing details are listed in Table A5-5. These studies dosed animals with a mixture of six NDL-PCBs (P28, P52, P101, P138, P153, and P180) that was identified by the researchers as environmentally relevant based on presence in fish matrices. Studies that examined the NDL-PCB mixture were all conducted by the same group of researchers based in the Université de Lorraine. Neurodevelopmental effects reported in these studies are summarized in Table A5-6.

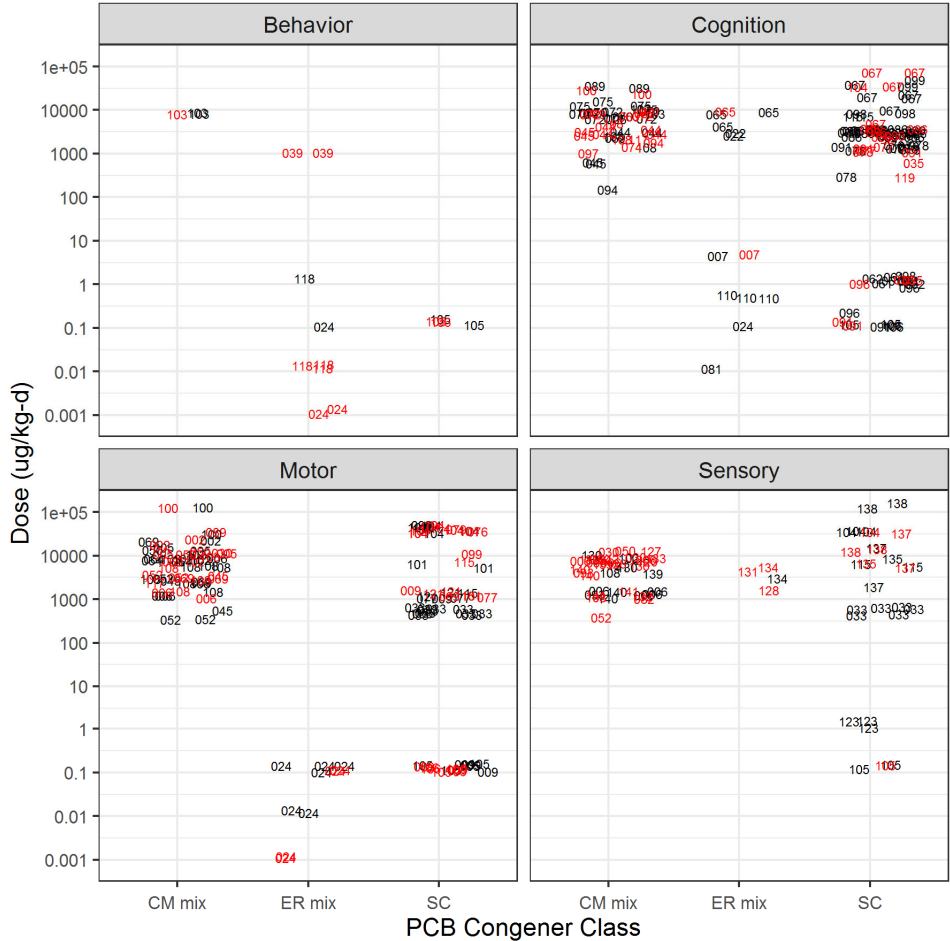


Figure A5-2. Effect levels identified in the rodent literature by neurodevelopmental endpoint Values correspond to unique study IDs assigned to each study in my database, and each point represents an individual test. Black and red coloring indicate NOAELs and LOAELs, respectively, for a given test. Study IDs are slightly offset both vertically and horizontally to allow for display of tests with the same MOEs. CM mix, commercial mixture (e.g., Aroclors); ER mix, environmentally relevant mixture (e.g., mimicking profile of contaminated fish, human breast milk, etc.); SC, single congener.

Table A5-5. Low dose oral rodent studies assessing neurodevelopmental effects of prenatal/lactational exposure to PCBs

Study	Species and Strain	Congener or Mixture	Doses (µg/kg-d)*	Period of Administration
Elnar <i>et al.</i> (2012)	Mouse, Swiss albino	P28, P52, P101, P138, P153, P180 (NDL mixture)	0.001, 0.010 or 0.100 (oral gavage)	PND0-21 (daily)
Elnar <i>et al.</i> (2016)	Mouse, Swiss albino	P28, P52, P101, P138, P153, P180 (NDL mixture)	0 or 0.010 (oral gavage)	PND0-21 (daily)
Dridi <i>et al.</i> (2014)	Mouse, Swiss Albino	P28, P52, P101, P138, P153, P180 (NDL mixture)	0.085, 0.216 or 0.400 (oral diet, in eels)	GD6-PND21 (daily)
Karkaba <i>et al.</i> (2017)	Mouse, CD1	P28, P52, P101, P138, P153, P180 (NDL mixture)	0.010 or 1 (oral diet)	GD6-PND21 (daily)

* Excluding controls

Table A5-6. Neurodevelopmental effects associated with prenatal/ lactational exposure to PCBs in environmentally-relevant rodent studies

Study (Species; Congener or Mixture)	Cognitive (<i>i.e.</i> , learning and memory) ($\mu\text{g/kg-d}$)	Behavioral (<i>i.e.</i> , response inhibition, anxiety) ($\mu\text{g/kg-d}$)	Motor function (<i>i.e.</i> , general activity and FOBs) ($\mu\text{g/kg-d}$)
Elnar <i>et al.</i> (2012) (Mouse; six NDLS)	Morris water maze (PND 268): NOAEL: 0.100 LOAEL: none	Elevated plus maze (PND 40): NOAEL: none LOAEL: 0.001 Light-dark box (PND 160): NOAEL: none LOAEL: 0.001 Tail suspension (PND 270): NOAEL: 0.100 LOAEL: none	Open-field test (PND 26): NOAEL: None LOAEL: 0.001 Negative geotaxis (PND 5-11): NOAEL: 0.010 LOAEL: 0.100 Forelimb grip strength (PND 5-11): NOAEL: 0.100 LOAEL: none WESPOC (PND 32): NOAEL: None LOAEL: 0.001
Elnar <i>et al.</i> (2016) (Mouse; six NDLS)	Y-maze maze (PND 413): NOAEL: 0.0100 LOAEL: none	NA	NA
Dridi <i>et al.</i> (2014) (Mouse; six NDLS in eels)	Y-maze (PND 38): NOAEL: 0.400 LOAEL: none Morris water maze (PND 120 & 123): NOAEL: 0.400 LOAEL: none	NA	NA
Karkaba <i>et al.</i> (2017) (Mouse; six NDLS)	NA	Sociability test (PND 50 & 330): NOAEL: None LOAEL: 0.010 Social novelty test (PND 50 & 330): NOAEL: None LOAEL: 0.010	NA

* To be conservative, LOAELs are reported in this table are based on the presence of at least one measurement noted as statistically significant for a test. These measurements are assessed in the text below for toxicological relevance.

NA - not applicable

Overall, studies that administered doses at levels environmentally relevant for the Spokane River (*i.e.*, ng/kg-d range) did not identify cognitive or motor effects in rodents. While Elnar *et al.* (2012) does note effects in

two tests of anxiety, these are not replicated in other studies at comparable or significantly higher doses and rely on a number of measurements that either do not show dose-response within the study or are not clearly clinically significant. Studies that administer levels greater than 1 mg/kg-d report mixed results on test of learning and spatial memory, as well as overall activity level and motor coordination. I discuss the neurodevelopmental outcomes reported in these studies below.

No effects were seen on cognitive (learning and memory tests) conducted on rodents at the lowest doses; studies dosing at 1 mg/kg-d or greater report mixed results. These tests showed the following:

- **Morris water maze:** Elnar *et al.* (2012) found no effects on PND 268 at doses of the six NDL-PCB mixture of 0.001, 0.010, and 0.100 µg/kg-d. Dridi *et al.* (2014) found no effects on PND 120 and PND 123 at doses of the six NDL-PCB mixture (from eels) of 0.085, 0.216, and 0.400 µg/kg-d.

Morris water maze testing on rats perinatally exposed to 0.100 µg PCB126/kg-d found no differences compared to controls (Vitalone *et al.*, 2008; Vitalone *et al.*, 2010).

In some studies at doses well above what would be expected for consumers of fish from the Spokane River, effects were reported in Morris water maze testing. For example, studies that dosed rats or mice perinatally with either single congeners or Aroclors at levels ranging from 1 to 6 mg/kg-d report effects including increased relearning latency (Eriksson and Fredriksson, 1996; Provost *et al.*, 1999; Sugawara *et al.*, 2006; Yang *et al.*, 2009). However, other studies report no effects at similar or higher doses (Donahue *et al.*, 2004; Eriksson and Fredriksson, 1996; Sugawara *et al.*, 2008; Zahalka *et al.*, 2001).

- **Y or T-maze:** Elnar *et al.* (2016) found no effects on PND 413 on mice dosed with 0.01 µg/kg-d of the six NDL-PCB mixture. Dridi *et al.* (2014) found no effects in a Y-maze test conducted at PND 38 on mice exposed to 0, 0.085, 0.216, or 0.400 µg/kg-d of the six NDL-PCB mixture (from eels).

Studies that dosed rodents at levels well above environmental relevance (0.100 µg/kg-d in the case of PCB126 and from 1 to 64 mg/kg-d in the case of commercial mixtures and other single congeners) identified mixed results. Several identified no differences compared to controls (Boix *et al.*, 2010; Schantz *et al.*, 1996; Schantz *et al.*, 1997; Tian *et al.*, 2011; Zahalka *et al.*, 2001), while other studies identified somewhat delayed acquisition that in some cases was transient or sex-specific (Boix *et al.*, 2010; Piedrafita *et al.*, 2008; Schantz *et al.*, 1995; Shiota, 1976).

With regard to behavioral endpoints, Elnar *et al.* (2012) reports some differences compared to controls in tests of behavioral signs of anxiety in mice administered the six NDL-PCB mixture at doses in the ng/kg-d range. However, few of the measurements noted a clear dose-response. Further, tests of sociability/novelty in mice exposed to the same six NDL-PCB mixture at comparable exposure levels identified no major differences with controls, and studies using the same behavioral test (elevated plus maze) to evaluate offspring of rodents dosed with significantly higher levels of PCBs did not replicate the Elnar *et al.* (2012) results. These tests showed the following:

- **Elevated plus maze:** Mice exposed to 0.001, 0.010, or 0.100 µg/kg-d of the six NDL-PCB mixture spent statistically significantly less time in open arms (2-3% vs. 6% in controls), however the absolute time was not reported and a difference of several percent does not appear meaningful. The middle and high dose groups showed an increase in latency on first entry into open arms (~140 s vs. 80 s for

controls) and an increase in number of attempts to enter open arms (10-12 attempts vs. 6 for controls) Elnar *et al.* (2012).

In mice exposed lactationally to 6 mg/kg-d Aroclor 1254, a level well above environmental relevance, no effects were seen in an elevated plus maze test conducted on PND 38 and 39 (Tian *et al.*, 2011). Rats perinatally dosed to PCB126 at 0.100 µg/kg-d also showed no major differences relative to controls in an elevated plus maze test at PND 100 and at 12 months of age, except for a slight increase in overall motor activity in males (Vitalone *et al.*, 2008; Vitalone *et al.*, 2010).

- **Light-dark box:** Mice exposed to 0.001, 0.010, or 0.100 µg/kg-d of the six NDL-PCB mixture had a greater time for first entry into the light box (100-130 sec vs. 50 sec in controls) (Elnar *et al.*, 2012). However, treated groups did not show a dose-response relationship. All three treatments showed significantly reduced time in the lit box (13-20 sec vs. 33 sec in controls), however the dose-response relationship was inverse. Low and middle dose groups showed a statistically significant difference compared to controls in the number of transitions between light and dark boxes, but the magnitudes were similar to controls (median of 1 vs. 3.5), and no significant difference was identified in the high dose group.
- **Tail-suspension:** No differences compared to controls were noted in mice exposed to 0.001, 0.010, or 0.100 µg/kg-d of the six NDL-PCB mixture (Elnar *et al.*, 2012). A similar test conducted on mice exposed to 6 mg/kg-d Aroclor 1248 also found no effects (Tian *et al.*, 2011).
- **Sociability/novelty:** Mice exposed to 0.010 or 1 µg/kg-d of the six NDL-PCB mixture showed no major differences compared to controls on PND 50 or PND 330 (Karkaba *et al.*, 2017). In the initial sociability test, male mice in the low dose group spent more time with the stranger than controls at PND 330, but no difference was noted in the high dose group. In the follow-up social novelty test, in which a second rodent was added to the empty chamber (in contrast to the now-familiar rodent in the first chamber), females in both dose groups spent less time than controls sniffing the familiar rodent on PND 50, however this was not seen on PND 330. These two measurements suggest heightened preference for novelty in the treated mice, however they are not corroborated by the other measurements in the tests.

In tests of motor activity, one open field test identified a reduction in horizontal (locomotor) activity in male offspring of mice administered a six NDL-PCB mixture at 0.001 and 0.010 µg/kg-d, but not at 0.100 µg/kg-d Elnar *et al.* (2012), in contrast to open field tests conducted in rodents dosed at or above 1 mg/kg-d that report either increased levels of locomotor activity or no differences compared to controls. FOB testing of gross and fine motor control identified no consistent effects at environmentally relevant levels, and mixed effects at higher doses. These tests showed the following:

- **Open field:** Mice exposed to 0, 0.001, 0.010, or 0.100 µg/kg-d of a six NDL-PCB mixture noted a decrease in horizontal motor activity in males in the 0.001 and 0.010 µg/kg-d dose groups; no effect was noted in females at any dose or in males at 0.100 µg/kg-d (Elnar *et al.*, 2012). No differences in rearing activity were noted. Without a clear dose-response identified, it is uncertain whether the effect noted can be attributed to PCB exposure.

- In some studies, open field tests conducted on rodents dosed well above environmental levels have noted higher activity levels when compared to controls (Sugawara *et al.*, 2006; Sugawara *et al.*, 2008; Tian *et al.*, 2011), while others have found no differences (Boix *et al.*, 2010; Haave *et al.*, 2011; Lilienthal *et al.*, 1990; Sugawara *et al.*, 2006).
- **FOB:** Elnar *et al.* (2012) assessed mice exposed to 0, 0.001, 0.010, or 0.100 µg/kg-d of a six NDL-PCB mixture via several FOB tests of motor strength and coordination. Transient effects were noted in female mice in the 0.1 µg/kg-d group in the negative geotaxis test, disappearing by PND 11; no effects were noted in males. No effects were noted in any dose group on tests of grip strength from PND 5-11. In a WESPOC test conducted at PND 32, males in the 0.001 and 0.100 µg/kg-d dose groups, but not the middle 0.010 µg/kg-d dose group, were noted to have a greater pole grasping latency. No effects were seen in females, and no effects were seen in males in swimming quality, climbing time, or escape latency.

FOB tests conducted at higher doses of single congeners or Aroclors do not identify any clear dose response. Grasp and righting reflex tests conducted on rats exposed to 0.1 µg/kg-d PCB126, well above environmental relevance, showed no effects (Vitalone *et al.*, 2008). Two rotarod tests conducted at the same dose level report contradictory results (Cauli *et al.*, 2013; Vitalone *et al.*, 2008), and a negative geotaxis test conducted on rats exposed to 0.1 µg/kg-d PCB126 showed effects (Vitalone *et al.*, 2008). Results are similarly mixed for rodents dosed with Aroclors or other congeners above 1 mg/kg-d. Effects were found in FOB tests conducted on rodents dosed 1 to 375 mg/kg-d (Boix *et al.*, 2010; Kuriyama and Chahoud, 2004; Nguon *et al.*, 2005; Tilson *et al.*, 1979), however FOB tests are reported as negative in doses ranging from 1 to 18 mg/kg-d in other studies (Boix *et al.*, 2010; Haave *et al.*, 2011; Nguon *et al.*, 2005; Roegge *et al.*, 2004; Sitarek and Gralewicz, 2009; Sugawara *et al.*, 2008).

While I closely assessed the results of the Université de Lorraine laboratory as they administered mice a mixture NDL-PCBs at environmentally relevant dose levels, I note a number of important methodological issues with their approach. For example, Elnar *et al.* (2012) and Elnar *et al.* (2016) administered Swiss albino mice a NDL-PCB mixture at dose levels of 0.001, 0.010, or 0.100 µg/kg-d in 10 mL/kg rapeseed oil via oral gavage. Control animals received the vehicle, *i.e.*, rapeseed oil, only. At these low delivered doses, the potential contribution of background sources to total doses must be considered. For example, Roszko *et al.* (2012) and Wroniak and Rekas (2017) report concentrations of the same six NDL-PCB compounds, as well as other contaminants, in rapeseed oil samples. Reported concentrations of the six NDL-PCB mixture in rapeseed oil samples range from 443.9 pg/g (Roszko *et al.*, 2012). to 2,599.4 to 8,380.8 pg/g Wroniak and Rekas (2017). Rapeseed oil has a mean density of 0.9133 g/ml at room temperature (Sahasrabudhe *et al.*, 2017) Elnar *et al.* (2012) administered 10 mL/kg of rapeseed oil per day containing these concentrations, yielding a contribution of rapeseed oil to the total dose of these six congeners ranging from 4.05 (based on the Roszko *et al.* (2012) measurements) to 23.74-76.54 ng/kg-d (based on the Wroniak and Rekas (2017) measurements). At these background levels, actual administered doses of these congeners at the lowest one or two doses administered the Elnar studies (Elnar *et al.*, 2016; Elnar *et al.*, 2012); (0.001 ng/kg and 0.010 µg/kg-d) would have been essentially equal to doses administered to control animals (Table A5-7). However, based on the information provided by the Elnar studies (Elnar *et al.*, 2016; Elnar *et al.*, 2012), actual doses received by the animals were not verified by subsequent analysis of dose solutions.

Table A5-7. Estimated total doses of six NDL-PCB congeners delivered to mice in Elnar *et al.* (2016); Elnar *et al.* (2012), assuming addition of background concentrations in rapeseed oil reported by Roszko *et al.* (2012) and Wroniak and Rekas (2017) to target administered doses

Target NDL-PCB dose (ng/kg-d)	Estimated total NDL-PCB dose with addition of background dose from rapeseed oil (ng/kg-d)	
	Based on Roszko <i>et al.</i> (2012)	Based on Wroniak and Rekas (2017)
0	4.05	23.74-76.54
1	5.05	24.74-77.54
10	14.05	33.74-86.54
100	104.05	123.74-176.54

In addition, the dose levels used by Elnar *et al.* were achieved by serial (1:10) dilution of a stock solution containing 10 mg of NDL-PCB mixture in 10 mL of rapeseed oil. To achieve the target doses of 0.100, 0.010, and 0.001 µg/kg, four to six dilutions would have been required, with the lowest 0.001 µg/kg dose level requiring a 1,000,000-fold dilution relative to the stock solution. At the very low levels in resulting dose solutions, error in dose propagation resulting in significant differences from target dose levels could occur, however again no confirmation of the actual dosed levels was provided.

Dridi *et al.* (2014) dosed mice with a food paste containing mixtures of ground river and sea eel that were analyzed for the presence of the six NDL-PCBs. Since the eels likely contained other contaminants, including additional PCBs, it is likely that the mice were also exposed to additional contaminants (the study noted the presence of mercury and heavy metals in the eels). Further, the three dose groups included in the study were not administered a homogenous eel mixture: the 0.085 µg/kg-d dose was derived from sea eels only, the 0.216 µg/kg-d dose was derived from a mixture of sea and river eels, and the 0.400 µg/kg-d group was derived from river eels only. Thus, it is likely that the type and relative amount of contaminants differed between the dose groups. Control animals were administered the same food paste minus the eel. The study does not indicate that dose levels in the control or PCB groups were confirmed by subsequent analyses.

E. Exposure assessment

For a general description of the exposure assessment methodology (*e.g.*, data sources, fish species, sampling locations, assumptions) by which I estimate PCB intake rates (ng/d) for consumers of fish from the Spokane River, see Section II.A and Appendix 2. I used these data to derive PCB congener- or mixture-specific consumption rates, as I describe below.

PCB intake rates were estimated for two different scenarios: 1) a best estimate, using mean PCB tissue concentrations and mean fish consumption rates (FCRs) and 2) a reasonable upper bound, based on the maximum of either the mean PCB tissue concentrations and 95th percentile FCRs or the 95th percentile PCB tissue concentrations and mean FCRs. The upper bound estimates for FCRs and tissue concentrations are highly conservative because they are based on the sum of the 95th percentile estimates of FCRs or tissue concentrations determined across individual species. For example, the upper bound FCR scenario assumes that a person eats every species of fish at a rate equal to the 95th percentile consumption rate for each species, while the upper bound tissue concentration scenario assumes that every species of fish that a person eats contains PCBs at a concentration equal to the 95th percentile concentration for the species.

First, I estimated total intake rates for individual congeners based on species-specific tissue concentrations and species-specific intake rates for rainbow trout, largemouth bass, and mountain whitefish caught in the Spokane River (see Appendix 2). Note that while I included all congeners in the exposure assessment, I only considered congeners administered in neurodevelopmental or reproductive animal studies (see Table A5-8) when determining MOEs (see Section IX.F, beginning on p. 307). Because individual congeners were in some cases also reported as coelutions (*e.g.*, PCB153/168), I developed a rubric to assign individual congener intake rates. For congeners reported *only* as coelutions (*e.g.*, PCB53 reported as PCB153/168), I conservatively assumed 100% of the PCB intake rate to be the target congener (*i.e.*, PCB153). If a congener was reported both as a single elute and as a coelute (*e.g.*, PCB183 and PCB183/185), I used the maximum of the two intake rates.

To derive PCB intake rates for mixtures of congeners used in toxicological studies, I summed the intake rates of each individual congener composing the defined mixture. For example, the intake rate of a mixture of PCB101 and PCB118 would be the sum of each of their individual intake rates mentioned above. I also assumed that the relative percentage of each congener in the mixture was the same as that in the mixtures used in the animal studies.

Lastly, total PCB intake rates were calculated independently of the individual congener analyses to avoid overestimating total PCB intake rates. Coelutions were reported consistently within a given sample (*i.e.*, fish fillet) but inconsistently across the PCB tissue dataset; thus in order to avoid double-counting the intake rates for congeners reported both individually and as coelutes, total PCBs were calculated by taking, for each species, the sum of congeners for each relevant sample in the dataset and then taking the average of these sums across species. Since the precise composition of Aroclors, Kanechlors, and other commercial mixtures used in the toxicological studies was not reported, I assumed that the intake rate of PCBs (ng PCB/d) associated with these mixtures was equivalent to the total PCB intake rate.

Estimated total PCB intake rates for the upper bound exposure scenario described above were higher when using mean tissue concentrations and 95th percentile FCRs (1.115 µg/day) than the 95th percentile tissue concentrations and mean FCRs (0.417 µg/day); consequently, I conservatively used the higher estimate in my calculations for the upper bound exposure scenario. Intake rates were normalized by human body weight (a default body weight value of 80 kg was applied per US EPA guidelines (US EPA, 2011a), to yield total PCB intake rates of 0.003 and 0.014 µg/kg-d for the best estimate and reasonable upper bound scenarios respectively.

Table A5-8 lists the estimated congener- or mixture-specific consumption rates. I used these consumption rates in the MOE calculations in Section F.

Table A5-8. Estimated PCB intake rates for consumers of fish from the Spokane River. Intake rates are presented for two scenarios: a best estimate and a reasonable upper bound (see text). Only congeners or mixtures administered in the neurodevelopmental or reproductive animal studies (see Appendix 6 for assessment of reproductive effects) were included in this analysis.

PCB congener or mixture	PCB intake rate by scenario (ng/kg-d)	
	Best estimate	Reasonable upper bound
PCB4	0.0000978	0.000419
PCB15	0.000159	0.000563
PCB28	0.0299	0.132
PCB47	0.0697	0.308
PCB52	0.0828	0.373
PCB74	0.166	0.736
PCB77	0.00465	0.0206
PCB80	0	0
PCB95	0.0174	0.0870
PCB118	0.217	0.979
PCB126	0.000574	0.00272
PCB132	0.0255	0.110
PCB138	0.140	0.581
PC153	0.200	0.902
PCB156	0.0248	0.113
PCB169	0.0000159	0.0000523
PCB180	0.109	0.482
PCB28, 52, 101, 138, 153, 180	0.733	3.25
PCB28, 52, 101, 118, 138, 153, 180	0.951	4.23
PCB28, 77, 101, 105, 118, 126, 138, 146, 153, 156, 169, 170, 180, 187	1.13	5.03
PCB47, 77	0.0743	0.328
PCB52, 66, 74, 105, 118, 138, 153, 156, 157, 180, 183, 187, 189, 194, 203	1.30	5.75
PCB74, 94, 99, 118, 126, 138, 153, 156, 169, 170, 180, 187, 194, 200	1.16	5.19
PCB77, 169	0.00466	0.0206
PCB77, 105, 118, 126, 138, 153, 169, 180	0.755	3.34
PCB101, 118	0.389	1.76
PCB118, 126	0.218	0.981
PCB126, 153	0.200	0.904
Aroclors or other commercial mixtures	3.11*	13.9*

* Intake rates for Aroclors and other commercial mixtures were considered to be equivalent to total PCB intake rates.

F. Risk characterization

In this section, I integrate the data from the hazard, dose-response, and exposure assessments described above to assess the risk of PCB exposure to consumers of fish from the Spokane River. To do so, I use a Margin of Exposure (MOE) approach, whereby the point of departure (*i.e.*, NOAEL or LOAEL) for a given neurodevelopmental test in animals (adjusted to a human-equivalent dose (HED) according to body size scaling factors³⁶ per US EPA (US EPA, 2011d) is compared to the estimated PCB consumption rate for that congener or mixture of congeners by consumers of fish from the Spokane River. The general formula used to calculate the MOE for a given PCB congener or mixture is as follows:

$$MOE = \frac{NOAEL \text{ or } LOAEL \text{ (animal test)} \left(\frac{mg}{kg-d} \right) \times \frac{1}{HED \text{ scaling factor}}}{PCB \text{ consumption rate} \left(\frac{mg}{kg-d} \right)}$$

MOEs represent the “established safety buffer between the toxicity effect dose level and the predicted exposure dose” (US EPA, 2012b). The lower the MOE, the more likely a chemical is to pose an unreasonable risk; for a chemical with an MOE<1, the exposure dose or concentration is greater than the scaled NOAEL or LOAEL. According to US EPA, an acceptable MOE for LOAEL-based assessments is 1,000 (US EPA, 2012b). A value of 1,000 reflects consideration of standard uncertainty factors applied in risk assessment to extrapolate from the point of departure to reference dose levels (*e.g.*, to extrapolate between species, account for intraspecies variation, and extrapolate from a LOAEL to NOAEL, where each uncertainty factor is assigned a value of 10). For a NOAEL-based assessment, an acceptable MOE would be 100.

Note that MOEs in this assessment are based on highly conservative PCB intake rate estimates. The upper bound estimates are based on the sum of 95th percentile estimates of FCRs and mean tissue concentrations determined across individual fish species. In other words, the upper bound FCR scenario assumes that a person eats every species of fish at a rate equal to the 95th percentile consumption rate for each species.

Figure A5-3 presents the calculated MOEs for all neurodevelopmental tests in the 87 studies passing my database filtering criteria prior to implementing an MOE threshold cutoff (see Table A5-1). MOEs for both the best estimate (Figure A5-3A) and reasonable upper bound (Figure A5-3B) scenarios are provided. The MOEs are presented by species and neurodevelopmental endpoint, as well as by whether the effect seen in the animals was a NOAEL or LOAEL. Because most of the animal neurodevelopmental studies I identified exposed animals to PCB doses well above environmental exposure levels, the majority of calculated MOEs for both scenarios are at or above 1,000 and in most cases are orders of magnitude higher than 1,000.

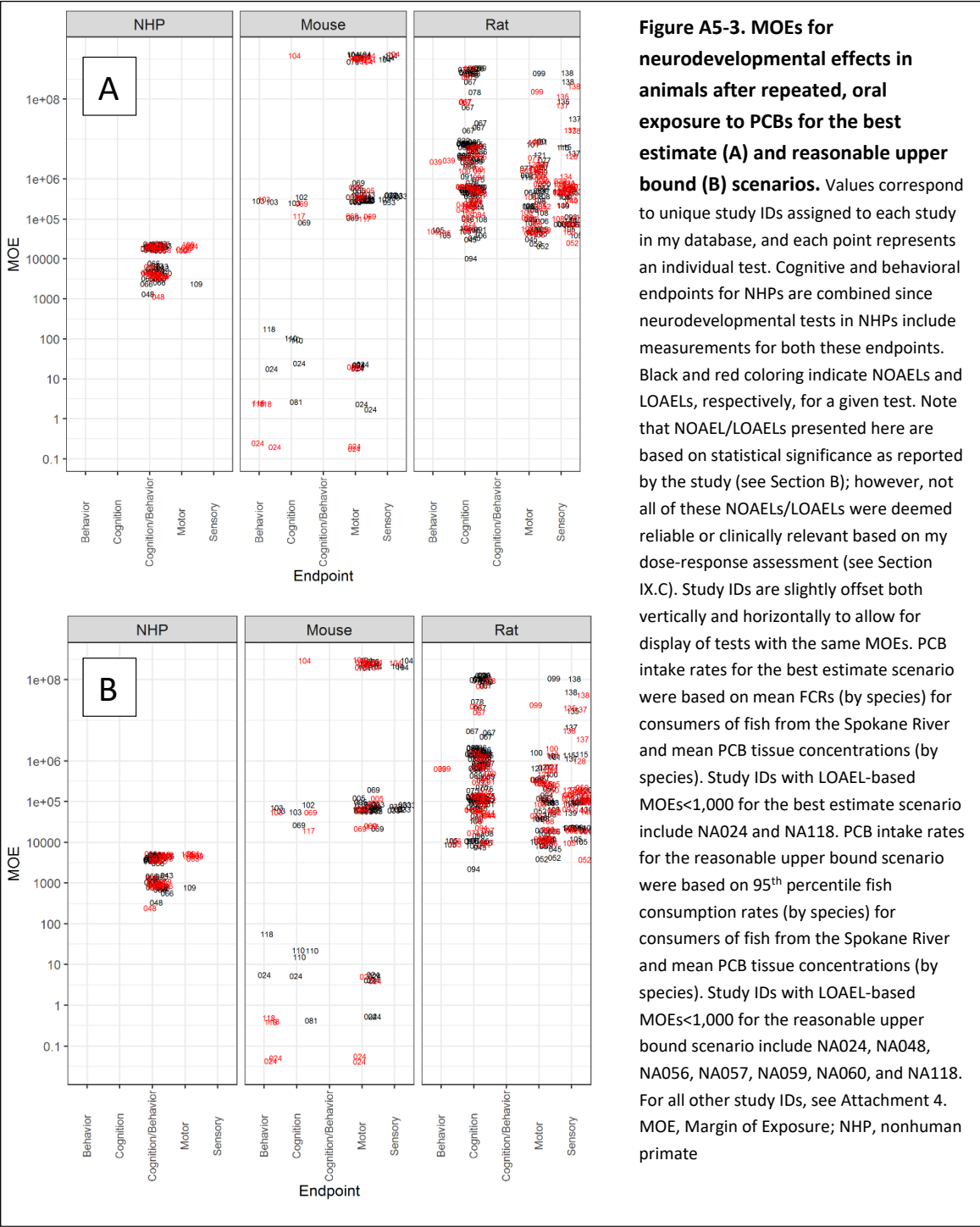
Of the 87 animal studies I reviewed that passed the screening criteria, for the best estimate scenario, four yielded an MOE<1,000 even in the absence of effects, that is, even if a NOAEL is used in the calculation (Figure A5-3). These MOEs correspond to doses in the ng/kg-d to low µg/kg-d range. All four studies were in mice. Of these four studies, two identified at least one LOAEL for a neurodevelopmental test with an MOE<1,000 (MOEs ranging from 0.181 to 18.1). For the reasonable upper bound scenario, 11 studies yielded an MOE<1,000 (inclusive of both NOAELs and LOAELs in the calculation), four of which were in mice and seven in NHPs. Of these 11 studies, seven identified at least one LOAEL for a neurodevelopmental test with

³⁶ Species-specific scaling factors for HED calculations are: NHP = 1.7, mouse = 7.5 and rat = 4.2 (US EPA, 2011a).

an MOE<1,000 (MOEs ranging from 0.0410 to 753). NOAEL/LOAELs were based on statistical significance as reported by the study (see Section C); however, not all of these NOAELs/LOAELs were deemed reliable or clinically relevant based on my dose-response assessment (see Section D).

These seven studies are described in detail in Section D.2 and D.3 alongside the rest of the literature for potential relevance to effects on learning and memory, behavioral effects including anxiety and response inhibition, and generalized activity. Overall, results from these studies were found to lack replicability due to the wide variability in experimental design, show no or slight differences between treated animals and controls, and exhibit inconsistent dose-response and/or effects not seen in similar testing conducted at higher doses (*i.e.*, MOE>1,000).

Comparison of predicted exposure levels to US EPA reference doses (RfDs) for Aroclors further suggests that no adverse noncancer effects are expected. An RfD is defined as an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily oral exposure for a chronic duration (up to a lifetime) to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime (US EPA, 2019d). US EPA has set oral RfDs for noncancer endpoints for Aroclor 1016 and Aroclor 1254 of 0.07 and 0.02 µg/kg-d, respectively. Neither of the RfDs for these two Aroclors are based on neurodevelopmental effects; the RfD for Aroclor 1016 is based on reduced birth weights in monkeys and RfD for Aroclor 1254 is based on immune, dermal and ocular effects in monkeys (US EPA, 1993, 1994). As shown in Table A5-8, the estimated upper bound Spokane River consumption rate is 0.014 g/kg-d. Therefore, comparison to the RfDs suggests Aroclors are not likely to cause noncancer effects.



The assessment I conducted makes conservative assumptions about the amount of fish consumed from the Spokane River. Using conservative assumptions about exposure is one (of many) ways a toxicologist can ensure that risks estimated in a human health risk assessment are not underestimated. In this assessment, for the reasonable upper bound estimate, I assume that every day, a person consumes each species of fish caught from the Spokane River at a rate that is near the maximum consumption rate for all people who catch and consume this fish (*i.e.*, at the 95th percentile of the consumption rate for each species of fish). This scenario is not plausible; however, I use this conservative consumption rate in my assessment to calculate the margins of exposure (MOEs) for each study I identify as relevant. Thus, the reader should consider the level of conservatism when assessing the results of this toxicological assessment

Based on this assessment of the hazard, exposure, and toxicity of PCBs, and using a weight-of-evidence approach, I conclude that it is highly unlikely that any adverse neurodevelopmental outcomes will occur from exposure to PCBs in fish caught and consumed from the Spokane River.

X. Appendix 6: Reproductive toxicology of PCBs

A. Introduction to risk assessment for reproductive outcomes

PCBs are commonly measured environmental contaminants. As a class, PCBs have been reported to cause health effects in animals including effects on reproduction and development. The endpoints reported with exposure to PCBs include reduced fertility in males and females, abnormal menstrual cycling in females, reduced birthweight in offspring, and increased incidence of still birth (Ahmad *et al.*, 2003; Arnold *et al.*, 1995). Males have reported changes in semen quality parameters (sperm concentration, motility, and morphology), sperm DNA integrity (DNA damage or chromatin fragmentation), and circulating reproductive hormone levels (Meeker and Hauser, 2010).

I conducted a toxicological assessment of the animal literature to assess whether intake of PCBs from fish by consumers of fish from the Spokane River is likely to cause adverse reproductive and developmental effects. My approach follows a standard risk assessment framework, including hazard identification, dose-response assessment, exposure assessment, and risk characterization (Faustman and Omenn, 2013). For the purposes of this assessment, reproductive effects are those in parental animals that affect fertility or the ability to complete a successful pregnancy to birth. Developmental effects are those that affect the offspring's ability to thrive and reach reproductive age. I recognize these classifications might be defined differently by others. Neurodevelopment, specifically, is not covered in this appendix and is reviewed in Appendix 5.

B. Literature search approach

To identify reproductive and developmental toxicity studies relevant for my risk assessment, I conducted a review of the animal literature. My emphasis was on primary literature (*i.e.*, no review articles, meta-analyses, *etc.*); however, I did review reports from authoritative bodies such as the Agency for Toxic Substances and Disease Registry (ATSDR), United States Environmental Protection Agency (US EPA), *etc.*, if available. Combinations of search terms³⁷ were used to query PubMed and Google Scholar for studies assessing reproductive and developmental effects of PCBs in animals.

A total of 116 studies were identified based on these search terms. To support the dose-response assessment, I compiled relevant information for each study including species (and strain), exposure route, exposure frequency and duration, administered dose(s)³⁸, PCB congener(s) or mix, age at assessment, test(s), and endpoint(s). For each test within a study, I identified a no observed adverse effect level (NOAEL) and/or a lowest observed adverse effect level (LOAEL) based on reported statistical significance: an administered dose was identified as a LOAEL if an effect was statistically significant at a p -value <0.05 when compared to

³⁷ Search terms included: "polychlorinated biphenyl" AND "reproductive" OR "PCB" AND "reproductive" OR "polychlorinated biphenyl" AND "reproduction") OR "PCB" AND "reproduction" AND "rat" OR "rats" OR "mouse" OR "mice" OR "monkey*" OR "hamster*" OR "pig" OR "pigs" OR "piglet*" OR "sheep" OR "cow" OR "cows" OR "rabbit*"; limited to English language only. An update to the literature review was conducted on October 16, 2019 with the search terms: "polychlorinated biphenyl" AND "reproductive" OR "PCB" AND "reproductive" OR "polychlorinated biphenyl" AND "reproduction") OR "PCB" AND "reproduction" AND "rat" OR "rats" OR "mouse" OR "mice" OR "monkey*"; again limited to articles in English.

³⁸ When a study reported the administered dose in terms of quantity in feed, I derived a body weight dose (mg PCB/kg body weight per day) based on food consumption and body weight data provided by the study or default values from the literature. In addition, if dosing was intermittent instead of daily, I derived a daily dose based on exposure duration and frequency.

controls. If no statistical evaluation was conducted by the authors, NOAELs and/or LOAELs were not assigned. NOAELs and LOAELs were adjusted to a human-equivalent dose according to body size scaling factors as described in Section X.G (beginning on p. 332).

The literature review initially identified approximately 432 studies meeting the search term criteria. These studies were further filtered according to the criteria in Table A6-1 to generate a list of studies with relevance to the exposure scenario for the Spokane River (*i.e.*, PCB doses comparable to those estimated for consumers of fish from the Spokane River. Only animal studies that administered repeated oral doses of PCBs were considered (*i.e.*, no bolus dose studies). In addition, I only considered studies that used established animal models (*e.g.*, nonhuman primates (NHP), rats, or mice) and that dosed animals during pre-gestation, gestation, and/or lactation.

Table A6-1. Number of animal studies in database passing sequential criteria for evaluation of reproductive effects following exposure to PCBs

Criterion	No. of Studies
Reproductive studies*	432
<i>In vivo</i> studies with relevant reproductive endpoints†	111
Oral administration	71
Repeated dosing (<i>i.e.</i> , no bolus dosing)	61
Conducted relevant animal models‡	51
Additional studies from October 2019 search	5
MOE<1000 (<i>i.e.</i> , LOAELs at environmentally relevant doses)§	4

* Search terms for PubMed: "polychlorinated biphenyl" AND "reproductive" OR "PCB" AND "reproductive" OR "polychlorinated biphenyl" AND "reproduction") OR "PCB" AND "reproduction" AND "rat" OR "rats" OR "mouse" OR "mice" OR "monkey*" OR "hamster*" OR "pig" OR "pigs" OR "piglet*" OR "sheep" OR "cow" OR "cows" OR "rabbit*"; limited to English language only. "Development" was not specifically searched initially, but was later conducted with the addition of one additional study that met the above criteria. An update to the literature review was conducted on October 16, 2019 with the search terms: "polychlorinated biphenyl" AND "reproductive" OR "PCB" AND "reproductive" OR "polychlorinated biphenyl" AND "reproduction") OR "PCB" AND "reproduction" AND "rat" OR "rats" OR "mouse" OR "mice" OR "monkey*"; again limited to articles in English.

† Only reproductive and developmental endpoints included in US EPA guidelines were considered (US EPA, 1996). *In vitro* or mechanistic studies were excluded. Some of the excluded studies were not on polychlorinated biphenyls or were reviews of other studies.

‡ Monkeys, rats, or mice.

§ Based on 95th percentile of PCB intake rates for Spokane River fish consumers. According to US EPA, acceptable MOEs for NOAEL-based and LOAEL-based assessments are 100 and 1000, respectively. These safety buffers take into account uncertainty factors including interspecies and intraspecies variability (US EPA, 2012b).

C. Hazard identification

Since both dioxin-like (DL) and non-dioxin like (NDL) PCBs have been associated with reproductive or developmental effects in animals, I included studies that looked at both types of congeners and congener mixtures. The numbers of studies and congener/mixture types assessed in the filtered literature are presented in Table A6-2 by species. This section describes the types of reproductive hazards identified in animals, with information organized as follows: reproductive effects tested in females, reproductive effects

tested in males, developmental effects when both sexes were combined, developmental effects in female offspring, and developmental effects in male offspring.

Table A6-2. Number of studies represented by selected experimental parameters from the reproductive literature on PCB effects in animals[‡]

Parameter	NHP	Mouse	Rat
Effect			
Reproductive, female	11	12	13
Reproductive, male	1	3	4
Developmental, both sexes	0	7	15
Developmental, female	0	1	11
Developmental, male	0	4	13
PCB congener or mixture			
Single congener	1	4	9
Aroclors [*]	12	10	14
Environmental mixture [†]	0	3	3
PCB structural class			
Dioxin-like (DL)	1	5	7
Non-dioxin like (NDL)	0	3	4
DL + NDL	12	11	17

^{*} Other commercial mixes included (*e.g.*, Kanechlors, Clophens, *etc.*).

[†] Mixture formulated to include congeners present in environmentally relevant matrices (*e.g.*, human breast milk, contaminated fish, *etc.*).

[‡] Some studies are counted in multiple categories.

1. Reproductive effects in females

In NHP, the main reproductive effects reported were effects on conception and fetal death. The NHPs were exposed to mixtures of DL-PCBs; no NDL-PCBs were tested on NHP. Several studies report decreased conception rate and increased fetal death with Aroclor 1254 (Arnold *et al.*, 1995; Barsotti and Van Miller, 1984). In one study, a non-dose related change in progesterone levels was reported, but the authors concluded this was not treatment related (Truelove *et al.*, 1990). In another study, an increased length of estrous cycle was observed (Arnold *et al.*, 1993a), but the effect was not statistically significant (Truelove *et al.*, 1990).

In rats, both DLs and NDL/DL mixtures were reported to cause effects on fertility (Baker *et al.*, 1977; Jonsson *et al.*, 1975), estrous cycle (Brezner *et al.*, 1984), and body weight (Brezner *et al.*, 1984; Overmann *et al.*, 1987). With mixtures, the only reported effect was ovarian and uterine inflammation that did not occur with DL-PCBs alone (Yoshizawa *et al.*, 2009).

In mice, Tomza-Marciniak *et al.* (2014) reported a decrease in 17 β -estradiol with exposure to a NDL/DL mixture. Implantation rate was decreased with both NDL-PCBs and DL-PCBs (Marks *et al.*, 1981; Torok, 1976), but no effect was reported on gestation or litter parameters (Pocar *et al.*, 2012).

2. Reproductive effects in males

The available studies in male animals are few and generally evaluated only one relatively high dose in each study. In NHP, a decrease in testicular diameter was reported with exposure to Aroclor 1242 with no change in testosterone (Ahmad *et al.*, 2003). A study of DL-PCBs in rats reported no treatment-related effects except an increase in the ratio of testosterone to luteinizing hormone (LH) with no difference in either testosterone or LH alone (Desaulniers *et al.*, 1997). Decreases in sperm quality, testicular weight, and estradiol were reported in mice with exposure to Aroclor 1254 (Cai *et al.*, 2011).

3. Developmental effects in both sexes combined

In the NHP studies, an increase in neonatal death was reported, although the study populations were small (Arnold *et al.*, 1995; Barsotti and Van Miller, 1984). In a few rodent studies, offspring were evaluated collectively either for body weight or litter parameters (*e.g.*, litter size, sex ratio). In these studies, decreased offspring body weight was the only reported effect with exposure to Aroclor 1254 in rats (Brezner *et al.*, 1984; Sager, 1983; Yang *et al.*, 2009) and in mice with exposure to a NDL-PCB (McCoy *et al.*, 1995).

4. Developmental effects in females

Irregular estrous cycles (Meerts *et al.*, 2004; Sager and Girard, 1994) were reported in rats in adulthood following gestational exposure to Aroclor 1254. Delayed vaginal opening and age at first estrous were also reported with exposure to Aroclor 1254 (Brezner *et al.*, 1984; Sager and Girard, 1994; Yang *et al.*, 2009), as was decreased uterine weight at birth (Sager and Girard, 1994).

Pocar *et al.* (2012) presents the results of a three-generation mouse study with maternal exposure to a DL+NDL-PCB mixture and subsequent follow up of male and female offspring for three generations with no further exposure. This was the only study to report effects in female mouse offspring—the authors report a decrease in ovary weight and an increase in atretic follicles in F1 offspring only. No effects were reported in the F2 or F3 generations for females.

5. Developmental effects in males

In rats, a decrease in testosterone was reported in adult offspring with exposure to DL+NDL mixtures or DL-PCBs alone (Kaya *et al.*, 2002; Sager, 1983; Sugantha Priya *et al.*, 2017). In general, testicular and seminiferous tubule weight decreased in male offspring (Gellert and Wilson, 1979; Gray *et al.*, 1993; Kaya *et al.*, 2002; Sager, 1983; Sugantha Priya *et al.*, 2017; Yang *et al.*, 2009). A decrease in sperm count was reported with exposure to NDL-PCBs and DL+NDL mixtures, but not to DL-PCBs only (Faqi *et al.*, 1998; Gray *et al.*, 1993).

In studies that evaluated male offspring exposed prenatally to PCBs, no maternal effects were reported, but decreased testes weight, tubule diameter, and sperm viability were reported with exposure to a 50:50 mixture of PCB101 and PCB118 for generations F1 and F2; exposure was to only the parental generation (Fiandanese *et al.*, 2016; Pocar *et al.*, 2012). A decrease in sperm viability and type 1 to type 2 tubule ratio also was reported for generations F1, F2, and F3.

D. Mode of action

Several hypothesized modes of action (MOAs) are reported for reproductive and developmental effects of PCBs. These include effects on hormone levels, direct interaction with hormonal receptors or degradation of hormones, impairment of signaling pathways, oxidative stress, and reductions in thyroid hormone. PCBs can be categorized with regard to health effects in several ways, including based on planarity, molecular weight, homologs, or number of chlorine substitutions. Hypothesized MOAs for reproductive and developmental effects of PCBs are described below.

1. AhR-dependent MOA

DL-PCBs are thought to act through the arylhydrocarbon receptor (AhR) for some health endpoints, including reproductive effects. Consideration of reproductive and developmental effects observed in studies of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), a known AhR agonist that is known to act through the AhR for some health endpoints including reproductive effects (Figure A6-1), can provide insights into potential AhR-dependent MOAs for PCBs. Interaction with the AhR is the initiating event in an adverse outcome pathway (AOP) that leads to upregulation of specific genes, some of which are key in xenobiotic metabolism.

Studies using TCDD demonstrate that male offspring exposed during gestation are 100 times more sensitive than adult males to the development of effects on the reproductive system. TCDD decreases anogenital distance (AGD), accessory sex organ weights, and spermatogenesis (Birnbaum *et al.*, 1985). The critical period of exposure for TCDD effects on testis weight appears to occur before puberty; no effect on testis weight seems to occur if the exposure occurs during adulthood (Simanainen *et al.*, 2004). Based on studies in TCDD, male mice have decreases in spermatogenesis and other changes in normal sperm parameters that appear to be AhR-dependent. Studies in adult rats with altered AhR sensitivity report less change in sperm parameters compared to wildtype animals, but no difference in androgen (*e.g.*, testosterone) response. Thus, the effects on sperm parameters are not completely caused by decreased serum testosterone levels. The effects on adult accessory sex organ weights are related to decreases in testosterone concentrations and/or androgen responsiveness (Simanainen *et al.*, 2004).

Exposure of mice to TCDD reduces the number of ovarian follicles, leading to reduced peripheral estrogen concentrations. This effect is also shown with AhR null mice; *i.e.*, a 50% drop in the number of pre-antral/antral follicles was observed in the ovaries of deficient mice at 53 days of age, suggesting a non-AhR mechanism (Hutz *et al.*, 2006).

It is plausible that the AhR may have an impact on reproductive and developmental outcomes associated with exposure to DL-PCBs. However, an AhR-dependent mechanism is not fully supported for the effects reported in studies of NDL-PCBs, suggesting that non-AhR mechanisms are possible for both DL- and NDL-PCBs. For DL-PCBs, there is some evidence of involvement of other mechanisms (*e.g.*, impairment of calcium homeostasis, changes in PKC signaling, changes in mRNA levels; Danish Ministry of the Environment (2014); Kietz and Fischer (2003). In addition, some modes of action that also occur with NDL-PCBs (*e.g.*, effects on thyroid homeostasis, induction of oxidative stress) might ultimately arise from AhR-related mechanisms when they occur with DL-PCBs (Danish Ministry of the Environment, 2014).

2. Sex hormone effects

Low-molecular-weight PCBs have been reported to elicit estrogenic activity both *in vitro* and *in vivo*, while NDL-PCBs have been reported to be antiestrogenic (Pliskova *et al.*, 2005). Several mechanisms that support reported effects of PCBs on hormonal levels, and that would further have an effect on reproductive endpoints, have been proposed (Figure A6-1; Pliskova *et al.* (2005)). DL-PCBs may affect cytochrome P450 levels that can degrade estrogens in the blood and may have an effect on steroidogenesis. NDLs can also affect cytochrome P450 levels and have antiestrogenic activity.

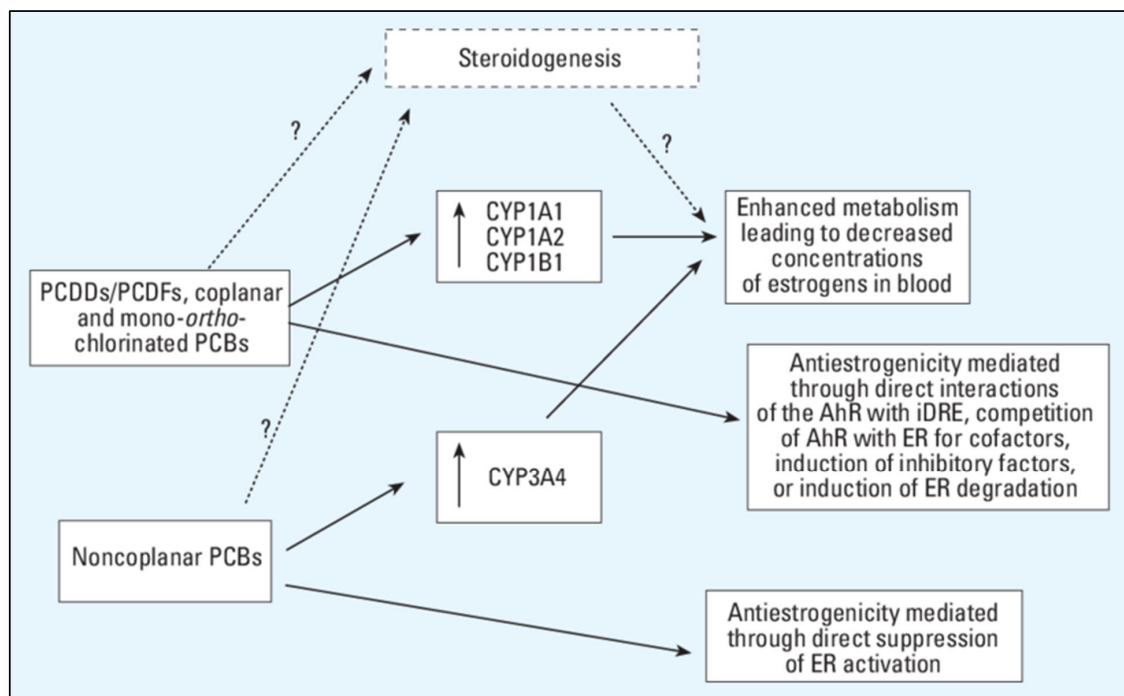


Figure A6-1. Potential MOAs for TCDD and PCBs (Pliskova *et al.*, 2005). AhR: arylhydrocarbon receptor, CYP: cytochrome, ER: estrogen receptor, iDRE: inhibitory dioxin response element, PCDDs/PCDFs: polychlorinated dibenzodioxins/polychlorinated dibenzofurans

Both estrogenic and anti-estrogenic activity of DL- and NDL-PCBs have been observed (Danish Ministry of the Environment, 2014; DeCastro *et al.*, 2006) including estrogenic effects such as increases in uterine weight and changes in estrogen and progesterone receptors in females (EFSA, 2005; Fischer *et al.*, 1998; Li and Hansen, 1995).

Although estrogen is necessary for proper development in male offspring, exposure to estrogenic and antiandrogenic chemicals may lead to poor semen quality due to effects in the seminiferous tubules, Leydig cells, or Sertoli cells (Sikka and Wang, 2008). Since endogenous estrogen levels in males are considerably lower than in females, the estrogenic/anti-estrogenic impact of high levels of contamination could be more pronounced in males (Pliskova *et al.*, 2005). Few studies of exposure to PCBs have assessed male reproductive parameters; however, decreases in hormone levels (testosterone, LH, estradiol) and changes in

sperm parameters are reported with exposure to both DL-PCBs and DL+NDL mixtures, but not with NDL-PCBs. Although there are too few studies to reach a conclusion, these findings suggest that the male parental effects are AhR mediated.

Reduction in ovarian estrogen secretion is reported with TCDD (and possibly DL-PCB) exposure *in vivo* (in the rat, trout, and zebrafish) and *in vitro* (in human and rat and monkey ovarian fragments). For TCDD, this deficit is manifested in the disruption of the steroidogenic pathway, resulting in attenuated secretion of estrogen. *In vitro* work suggests this occurs through a direct local, *i.e.*, ovarian, effect, rather than an indirect effect on the pituitary or hypothalamus. While the biochemical effects can differ with respect to dose, duration, animal, or model system, the resultant inhibition of estrogen synthesis may be responsible for the reduced fertility of animals or humans exposed to dioxins in the environment (Hutz *et al.*, 2006). Other effects observed after administration of TCDD to pregnant dams include attenuated secretion of ovarian estrogen, reduced numbers of large preovulatory ovarian follicles, and altered expression of ovarian mRNAs for the estrogen receptor (ER) and AhR in female peripubertal rat pups (Hutz *et al.*, 2006).

3. Oxidative stress

PCBs may induce the formation of reactive oxygen species (ROS) causing localized oxidative stress. A potential MOA is shown in Figure A6-2 (Selvakumar *et al.*, 2013)—this shows a multifaceted mechanism in which PCBs cause oxidative stress by interacting with the AhR and upregulating cytochrome P450s that increase ROS through metabolism of substrates. In addition, PCBs can be directly hydroxylated to form a quinone or semiquinone and superoxide, although this occurs mainly in PCBs with less than four chlorines.

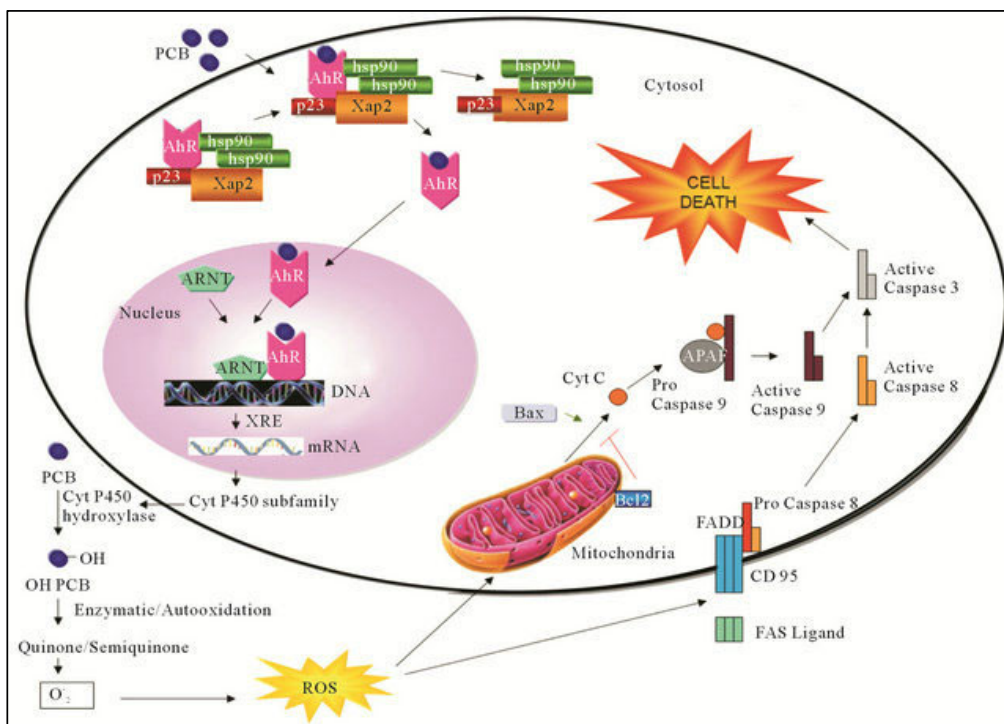


Figure A6-2. ROS generation with PCB exposure (Selvakumar *et al.*, 2013). AhR: arylhydrocarbon receptor, ARNT: AhR nuclear transporter, APAF: Apoptotic protease activating factor, Bax: Bcl-2-associated X protein, Bcl2: protein in apoptotic pathway, Cyt: cytochrome, FAS: apoptosis antigen 1, FADD: Fas-associated protein with death domain, hsp: heat shock protein, O_2^- : superoxide, ROS: reactive oxygen species, Xap: HBV X-associated protein 2, XRE: xenobiotic response element

4. Thyroid hormone effects

Effects of PCBs on thyroid hormone homeostasis have been documented, *e.g.*, as shown by decreased triiodothyronine (T3) and thyroxine (T4) concentrations in offspring of rats exposed to PCB153 from GD 10-16 (Kobayashi *et al.*, 2008; WHO, 2016). These changes in thyroid hormone homeostasis may impact reproduction and development. The interactions between thyroid hormones and estrogen are well documented. As shown in Figure A6-3 (Siegler *et al.*, 2012), both estrogen and thyroid hormones can interact with the estrogen response element (ERE) via multiple pathways. The activated estrogen receptor can stimulate the ERE to alter the transcriptional levels of key proteins involved in cell proliferation and survival (Siegler *et al.*, 2012).

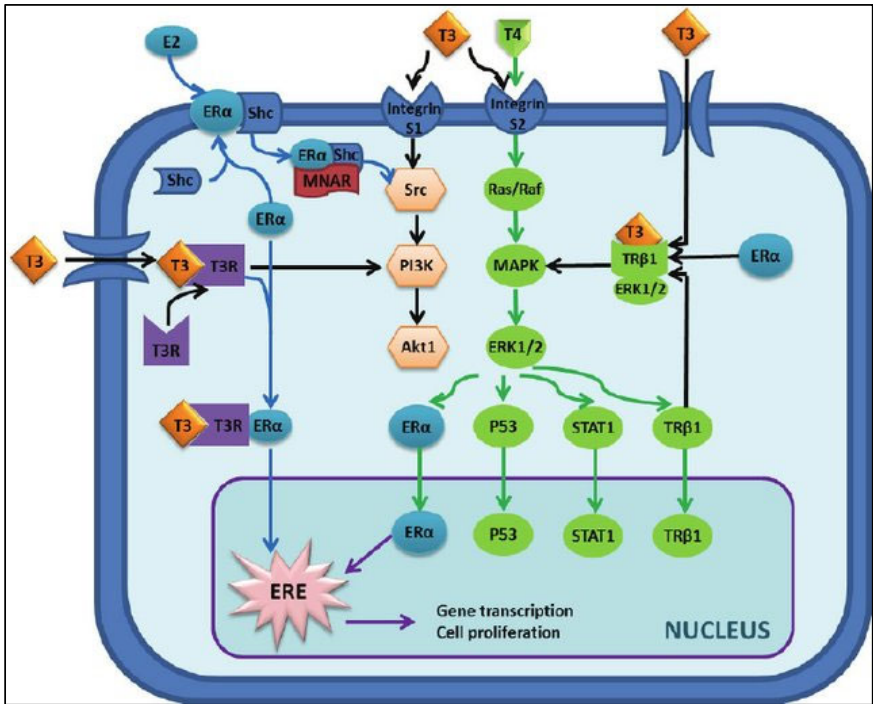


Figure A6-3. Interaction between thyroid hormone and estrogen (Siegler *et al.*, 2012). Akt1: protein kinase B, E2: estradiol, ER: estrogen receptor, ERE: estrogen response element, ERK1/2: extracellular signal-regulated kinases, MAPK: mitogen-activated protein kinase, MNAR: modulator of non-genomic activity of estrogen receptor, p53: a tumor suppressor protein, PI3K: Phosphoinositide 3-kinases, Shc; Src homology containing, T3R: T3 receptor, Src: a kinase, Ras/Raf: an activator and kinase, STAT1: signal transducer and activator of transcription, T3: triiodothyronine, T4; thyroxine, TRβ1: thyroid hormone receptor β1

5. Summary of MOA

The most plausible MOAs for PCBs leading to reproductive and developmental effects are through effects on sex and thyroid hormone homeostasis and oxidative stress. These MOAs may involve the AhR, but MOAs for reproductive and developmental effects are not solely mediated by the AhR, and may involve PXR and CAR-mediated induction of Cytochrome P450s 2B and 3A. The role of CAR and PXR in forming ROS, and modulating thyroid hormone levels, is discussed in Appendix 3. As with all responses, dose is a critical consideration, since doses that result in tissue concentrations below thresholds of activation will have no biological effects.

E. Dose-response assessment

In this section, I assess the relationship between dose of PCBs and incidence of reproductive or developmental effects in animals. I present my evaluation by male and female reproductive effects and then male and female developmental effects. While animal studies assessing reproductive or developmental effects associated with exposure to PCBs have been conducted in fish, birds, rodents, NHPs, and other

mammals, the focus of my assessment is on studies conducted in NHPs (which were all conducted in Rhesus monkeys), mice, and rats.

1. Female reproductive effects

Data on female reproductive effects are relatively robust: I identified 11 publications in NHPs, 12 studies in mice, and 13 studies in rats that met my literature search criteria and addressed female reproductive effects of PCBs. Female reproductive parameters that were reported in NHPs, rats, and mice include changes in body and organ weights, menstrual cycle parameters (NHPs only), hormonal levels, conception and gestation parameters, and litter parameters. Of note, multiple publications in NHPs provide information on female reproductive parameters, although some of these results are from the same cohort of animals and do not represent independent studies (*i.e.*, Allen and Barsotti (1976), Allen *et al.* (1980), and Barsotti *et al.* (1976) are the same cohort; Arnold *et al.* (1993a), (Arnold *et al.*, 1996), Arnold *et al.* (1996), and Truelove *et al.* (1990) are the same cohort). Table A6-3 summarizes information on effects that I considered to be potential reproductive effects resulting from exposure to PCBs, based on consistency of findings across species, reproducibility in multiple studies, and sufficient information on dose-response.

As shown in Table A6-3, the most sensitive female reproductive endpoints, based on the literature reviewed, was a reduction in conception in all three species and gestation (live births) in NHPs. A decrease in conceptions is consistently reported in the three species. The studies use a range of doses and additional endpoints are also evaluated. The range of NOAELs is 0.005 (Aroclor 1254) – 64 (individual congeners) mg/kg-d and the range of LOAELs is 0.02 (Aroclor 1254) – 12 (Aroclor 1242) mg/kg-d. A decrease in live births is reported in NHPs exposed to Aroclors with LOAELs ranging from 0.1 – 0.2 mg/kg-d; however, this effect is not reported in rodent studies at a range of doses.

Changes in body weights or reproductive organ weights were more frequently measured in rodent studies than in NHPs. Changes in body and organ weights are nonspecific endpoints; although changes in body weight may indicate systemic toxicity and have an indirect effect on reproductive and developmental endpoints due to a decrease in general health of the organism, body weight change is not generally considered to be reproductive endpoint. I do, however, include effects on this endpoint in Table A6-3 as this is a measure is commonly reported across many of the studies. Effects on weights of reproductive organs can be considered a reproductive effect; however, I observed no consistent effect on organ weights or histopathology sufficient to support identification of a point of departure (POD) based on this effect.

Specifically:

- Rats exposed to Aroclor 1254 or PCB153 did not have differences in body weight during gestation compared to controls (Baker *et al.*, 1977; Kobayashi *et al.*, 2008; Overmann *et al.*, 1987; Sager, 1983); one study reported a lower gestational body weight with exposure to 10 mg/kg-d Aroclor 1254, but this was the only dose tested (Brezner *et al.*, 1984). In rats, a lower body weight was reported among lactating females with exposure to 27 mg/kg-d Aroclor 1254; the difference was not reported at 2.7 mg/kg-d (Overmann *et al.*, 1987).
- In mice, maternal body weight differences were not apparent at any dose with exposure to Aroclor 1242, Aroclor 1254, a mixture of PCB101 and PCB118, PCB15, PCB153, PCB156, and PCB80; Aroclor 1242; or Aroclor 1254 (Birnbaum *et al.*, 1985; Fiandanese *et al.*, 2016; Fielden *et al.*, 2001; Marks *et*

al., 1981, 1989; Talcott and Koller, 1983; Welsch, 1985). A difference in weight gain was reported with exposure to PCB169 or PCB77 at 8 mg/kg-d and 16 mg/kg-d, respectively in mice (Marks *et al.*, 1981).

- Placental, ovarian, and uterine weights were unchanged with exposures of PCB156 up to 20 mg/kg-d in mice (Birnbaum *et al.*, 1985; Sanders and Kirkpatrick, 1977).

Functional and clinical changes in reproductive organs were also evaluated with little consistency in effect. In rats, a finding of uterine and ovarian inflammation was reported (Yoshizawa *et al.*, 2009) but not replicated in any other study and it is unclear how this single study in rats would relate to a human exposure. Findings of changes in reproductive organs include the following:

- An increase in endometriosis was not found with exposure of Aroclor 1254 at doses up to 0.08 mg/kg-d in NHPs (Arnold *et al.*, 1996).
- Exposure of rats to PCB153 or a mixture of PCB126 and PCB153 was associated with uterine and ovarian inflammation at 3 mg/kg-d for PCB153 alone and 0.003 mg/kg-d PCB126/3 mg/kg-d PCB153 for the mixture (Yoshizawa *et al.*, 2009). Exposure to a different mix of PCB118, PCB126, or the mixture of PCB118 and PCB126 did not cause inflammation at the highest doses tested in rats (Yoshizawa *et al.*, 2009). No increases in incidence of ovarian cysts or atrophy with any of the individual congeners or mixtures were reported with any dose tested (Yoshizawa *et al.*, 2009).

Increased menstrual cycle length in NHPs exposed to Aroclor 1254 or Aroclor 1248 was reportedly observed, but either subsequent statistical evaluation did not demonstrate that these findings were statistically significant or no study statistics were reported. Specifically:

- With exposure to Aroclor 1254, cycle length in NHPs was reported to be “erratic and longer” with exposure to 0.28 mg/kg-d (only dose tested; Arnold *et al.*, 1990) and increased in duration with exposure to 0.08 mg/kg-d (Arnold *et al.*, 1993a). Notably, Truelove *et al.* (1990) performed a statistical comparison (same cohort as Arnold *et al.* (1993a) and reported no significant difference in menstrual cycle duration. A separate study in NHPs exposed to 0.083 or 0.175 mg/kg-d Aroclor 1248 also reported an increase in cycle length that returned when PCBs were removed from the diet, although no statistics were provided (Allen *et al.*, 1980). Arnold *et al.* (1993a) and Truelove *et al.* (1990) reported no increase in anovulatory animals with Aroclor 1254 doses up to 0.08 mg/kg-d.

Hormonal changes were evaluated in NHP, rats, and mice. However, due to the inconsistency in response and the lack of available information presented in the few studies that evaluated hormonal levels, it is not justified to base a POD on hormone levels in NHP, rats, or mice. Specifically, studies showed:

- Changes in progesterone levels were reported to be decreased in NHPs with exposure to 0.005 mg/kg-d (the lowest dose tested) Aroclor 1254, with no change in estrogen (Truelove *et al.*, 1990). The authors noted “Since the statistically significant changes found in progesterone concentration did not occur in a dose related manner (the 20 and 40 µg/kg-d dose groups and all of test group 2 showed no differences) the toxicologic significance of this observation is equivocal.” A separate study in NHPs exposed to 0.083 or 0.175 mg/kg-d Aroclor 1248 also reported a decrease in

progesterone that returned to normal when PCBs were removed from the diet, although levels of progesterone and statistics are not provided (Allen *et al.*, 1980).

- In rats, a decrease in progesterone was reported in 36 week old but not 8 week old rats exposed to Aroclor 1242 at 6 mg/kg-d (Jonsson *et al.*, 1975).
- In mice, a decrease in 17 β -estradiol was reported with exposure to 0.7 mg/kg-d of a mixture of PCB28, PCB52, PCB101, PCB118, PCB138, PCB153, PCB180, but this was the only dose tested (Tomza-Marciniak *et al.*, 2014).

In studies assessing reproductive toxicity, the ability to conceive and carry an infant to birth can be measured in several ways; among these are to compare conception rates, implantation of fertilized eggs, gestation length, and number of live offspring between exposed and control animals. Although the results in studies in of PCBs in NHPs, rats, and mice, are varied based on dose, species, and exposure, a somewhat consistent response of a decrease in conception rate is observed. These results are summarized in Table A6-3. These studies show:

- In NHPs exposed to Aroclor 1016, 1248, or 1254, no difference was reported in ability to conceive (Allen *et al.*, 1980; Arnold *et al.*, 1990; Barsotti and Van Miller, 1984). The study by Arnold *et al.* (1995) exposed female NHPs to Aroclor 1254 in doses of 0, 0.005, 0.02, 0.04, or 0.08 mg/kg-d (n = 4/dose group). The animals were dosed daily by capsule for 25 months prior to mating with untreated males. A significantly greater incidence of fetal death compared to controls was reported when all the treatments groups were pooled, but when doses were examined individually, the difference was only significant for the highest dose group (0.08 mg/kg-d). A trend was also observed in dose-response, but this trend disappears if the control group is removed from the analysis, meaning there is no increase in severity with increase in dose. Conception was decreased in the 0.02, 0.04, or 0.08 mg/kg-d dose groups. Arnold *et al.* (1995) reported a negative trend for conception rate.
- Rats exposed to Aroclor 1242 had a decrease in conception (based on presence of vaginal plugs) with exposure to 12 mg/kg-d, but not 6 mg/kg-d (Jonsson *et al.*, 1975).
- In mice, a decrease in conception rate was reported with exposure to 12.5 mg/kg-d Aroclor 1254 (Welsch, 1985) and a lower percentage of pregnant mice with exposure to 750 mg/kg-d of PCB4 although no statistics were performed on this comparison (Torok, 1976). Most studies in mice report no change in implantations (Marks *et al.*, 1989; Orberg and Kihlstrom, 1973), but two report a lower percent of implantations in mice exposed to 64 mg/kg-d of PCB169 (Marks *et al.*, 1981) or 750 mg/kg-d PCB4 (Torok, 1976). Gestation was normal and no effects were observed on litter size, sex ratio, or viability index with up to 0.1 mg/kg-d of a mixture of PCB101 and PCB118 (Pocar *et al.*, 2012). Other studies reported no change in conception rate with 292 mg/kg-d Aroclor 1254 (Talcott and Koller, 1983); 16 mg/kg-d (Marks *et al.*, 1981); or 64 mg/kg-d of PCB15, PCB77, or PCB80 (Marks *et al.*, 1989).

Gestation length and perinatal viability were evaluated in all three species with no consistent effects reported. Studies in NHPs report a decrease in live births, but this outcome is not observed in rats and mice. These results are summarized in Table A6-3. These studies show:

- No difference in gestation length was reported with exposure up to 0.08 mg/kg-d of Aroclor 1254 in NHPs (Arnold *et al.*, 1995) or in rats exposed to 27.7 mg/kg-d Aroclor 1254 (Overmann *et al.*, 1987), but there was a reported increase in mouse gestation length with 375 mg/kg-d PCB4 (Torok, 1976).
- A decrease in the number of live offspring in NHPs was reported by Barsotti and Van Miller (1984) from 5 in the low dose group to 1 in the high dose group. Barsotti *et al.* (1976) also reported a decrease in live offspring at 0.095 mg/kg-d of Aroclor 1016.
- In rats, fewer dams brought a litter to term with 27 mg/kg-d of Aroclor 1254 than did controls (Overmann *et al.*, 1987). A decrease and delay in parturition was reported in dams exposed to 10 mg/kg-d of Aroclor 1254 (Brezner *et al.*, 1984). However, exposure to 10 mg/kg-d of Aroclor 1254, produced no effect compared to controls on litter parameters such as number of dead pups per litter at weaning, viable fetuses/litter, sex ratio, total number of newborn pups, and incidence of neonatal death (Brezner *et al.*, 1984).
- Similarly, in mice, no effect was reported on number of live fetuses per litter or fetal mortality with exposure to Aroclor 1254, PCB126, PCB153, or a mixture of PCB126 and PCB153 (Biegel *et al.*, 1989; Haake *et al.*, 1987; Mayura *et al.*, 1993; Morrissey *et al.*, 1992).

Table A6-3. Selected* findings on female reproductive parameters and dose ranges of PCBs (mg/kg-d)

Critical Effect	Species	Congener	Lowest Dose Tested	NOAEL Range	LOAEL Range	Highest Dose Tested	References
Body Weight	Rats	Aroclor 1254	0.26	2.7-43	27.7	64	Baker <i>et al.</i> (1977); Overmann <i>et al.</i> (1987); Sager (1983)
	Mice	Aroclor 1254	1	100-292	NA	292	Talcott and Koller (1983); Welsch (1985)
	Rats	PCB153	1	1-64	NA	64	Kobayashi <i>et al.</i> (2008)
	Mice	Individual congeners: PCB156, PCB169, PCB77, PCB15, PCB153, PCB80, PCB101/PCB118	0.001	0.001-50	8-64	62	Birnbaum <i>et al.</i> (1985); Fiandanese <i>et al.</i> (2016); Marks <i>et al.</i> (1981, 1989)
Conception	NHP	Aroclor 1254	0.005	0.005	0.02	0.08	Arnold <i>et al.</i> (1995)

Critical Effect	Species	Congener	Lowest Dose Tested	NOAEL Range	LOAEL Range	Highest Dose Tested	References
	Rats and Mice	Aroclor 1242, Aroclor 1254, CA60	1	6	1-12	12	Brezner <i>et al.</i> (1984); Jonsson <i>et al.</i> (1975); Orberg and Kihlstrom (1973)
	Rats and Mice	Individual congeners: PCB169, PCB77, PCB15, PCB80	0.1	4-64	4-8	750	Marks <i>et al.</i> (1981, 1989); Torok (1976)
Gestation and birth outcomes	NHP	Aroclor 1016, Aroclor 1242, Aroclor 1254	0.005	0.005-0.0297	0.02-0.2	0.2	Allen and Barsotti (1976); Arnold <i>et al.</i> (1995); Barsotti and Van Miller (1984); Litton Bionetics (1981)
	Rats and Mice	Aroclor 1254, PCB15, PCB169, PCB77, PCB80, Aroclor 1242, PCB126	0.001	0.003-75	NA	150	Brezner <i>et al.</i> (1984); Jonsson <i>et al.</i> (1975); Marks <i>et al.</i> (1981, 1989); Shirota <i>et al.</i> (2006); Welsch (1985)

* Summarizes information only on effects that are considered to be potential reproductive effects based on consistency across species, reproducibility, and sufficient information on dose-response. Other effects may have been reported in studies, but if not replicated in other studies or consistent with other literature, they were not included.

2. Male reproductive effects

The ability to discern a clear dose-response relationship for male reproductive effects is limited by the quantity and quality of available studies—I identified only eight publications that examined male reproductive effects, including one in NHPs and seven in rodents (some of these represent the same study cohort). These studies report findings for three primary male reproductive endpoints: organ (testes) weights, hormone levels, and sperm parameters.

As shown in Table A6-4, the male reproductive data are not sufficiently robust to determine a POD as few studies are available, a paucity of effects is reported, and there is little consistency across species. Based on the study by Cai *et al.* (2011), a POD for male reproductive effects equal to the NOAEL of 0.05 mg/kg-d (Aroclor 1254) can be identified in mice exposed to 0.0005, 0.005, 0.05, or 0.5 mg/kg-d every three days for

50 days. Alternatively, Cai *et al.* (2015) calculated a BMD and BMDL of 0.165 mg/kg-d and 0.016 mg/kg-d, respectively, for effects on sperm count, and a BMD and BMDL of 0.062 mg/kg-d and 0.0119 mg/kg-d for effects on sperm motility for Aroclor 1254 (Cai *et al.*, 2015).

Effects on reproductive organ weight or structure are reported in NHPs and rodents. These studies show:

- In NHPs, only one study provides information on male exposure to Aroclor 1242, but it is limited to a single dose (0.2 mg/kg-d for 6 months). The authors report a change in testicular diameter, but no change to testosterone levels or testes structure (Ahmad *et al.*, 2003). It is not justified to use this single study that tested only one dose as the basis for a POD for male reproductive toxicity.
- A single dose study in rats exposed to 50 mg/kg-d Aroclor 1254 for 7 days reported no effects on testes weight or structure (Dikshith *et al.*, 1975).
- In mice exposed to 0.0005, 0.005, 0.05, or 0.5 mg/kg-d Aroclor 1254 every 3 days for 50 days, a decrease in testes weight was reported in animals administered 0.5 mg/kg-d (Cai *et al.*, 2015).

Reproductive hormones measured in studies of PCBs in male NHPs, rats, or mice included estradiol, testosterone, LH, and FSH. As the only reported change was in estradiol levels in one study, these data are insufficient to use as the basis of a POD. These data are included in Table A6-4

There was no change to testosterone levels in NHPs exposed to Aroclor 1242 (Ahmad *et al.*, 2003). Other studies show:

- In male rats, Desaulniers *et al.* (1997) reports some changes in hormone levels. While rats exposed for 90 d to 0.0028, 0.036, 0.359, 3.783 mg/kg-d PCB28 or 0.00073, 0.0071, 0.075, 0.768 mg/kg-d PCB77 were reported to have no effects on FSH, LH, or testosterone at the highest dose tested for each congener, an increase in the testosterone to LH ratio was reported in rats exposed to the highest dose of PCB77 (Desaulniers *et al.*, 1997). However, the relevance of this finding is unclear.
- In C57 mice exposed to 0.0005, 0.005, 0.05, or 0.5 mg/kg-d Aroclor 1254 every 3 days for 50 days, a decrease in estradiol was reported in animals administered 0.5 mg/kg-d (Cai *et al.*, 2011).

Sperm parameters evaluated in studies of exposure to PCBs include viability, number of abnormal sperm, and sperm count. It is not justified to base a POD on the changes in sperm parameters alone as these were only reported to be changed in one study in mice and have not been replicated in mice or any other species. These data are summarized in Table A6-4. In C57 mice exposed to 0.0005, 0.005, 0.05, or 0.5 mg/kg-d Aroclor 1254 every 3 days for 50 days, a decrease in sperm count and increase in sperm mortality and abnormality was reported in animals administered 0.5 mg/kg-d (Cai *et al.*, 2011). While this study provides a consistent dose-response and could be used as the basis of a toxicity value, it is a single study in a single species (Table A6-4).

Table A6-4. Selected findings on male reproductive parameters and dose ranges of PCBs (mg/kg-d)

Critical Effect	Species	Congener	Lowest Dose Tested	NOAEL Range [†]	LOAEL Range [†]	Highest Dose Tested	References
Sperm parameters	Mouse	Aroclor 1254	0.0005	0.05	0.5	0.5	Cai <i>et al.</i> (2011)
Organ weights	Mouse	Aroclor 1254	0.0005	0.05	0.5	0.5	Cai <i>et al.</i> (2011)
Hormone Levels	Mouse	Aroclor 1254	0.0005	0.05	0.5	0.5	Cai <i>et al.</i> (2011)
	Rat	PCB28	0.0028	3.783	NA	3.783	Desaulniers <i>et al.</i> (1997)
	Rat	PCB77	0.00073	0.768	NA	0.768	Desaulniers <i>et al.</i> (1997)

* Summarizes information only on effects that are considered to be potential reproductive effects based on consistency across species, reproducibility, and sufficient information on dose-response. Other effects may have been reported in studies, but if not replicated in other studies or consistent with other literature, were not included.

[†] Only a single NOAEL or LOAEL is available rather than a range since only one study is available for each critical effect/species/congener. The study by Cai *et al.* (2011) included a control and four dose groups and the study by Desaulniers *et al.* (1997) had a control and single dose group only.

3. Male and female (combined) developmental effects

In the studies I reviewed, data for developmental endpoints, particularly effects on birth weights and malformations, are usually presented for both sexes pooled collectively. I identified 19 publications reporting on developmental effects, all in rodents.

Body weights of newborns are reported in all three species although there was no consistent pattern of response. Since the weight of evidence suggests no effect on body weight or malformations in rodents and change in body weight is only reported in one study in NHPs, it is not justified to base a POD on offspring body weight. As there was no consistent response, a table summarizing the NOAELs and LOAELs for this parameter is not included. These studies show:

- In NHPs exposed to 0.0297 mg/kg-d Aroclor 1016, infant weights were lower than controls (Barsotti and Van Miller, 1984). The authors state "... at weaning, the infant weights from the 1.0 ppm PCB group remained lower (864 ±97g) although not significantly different than controls (896± 90g)."
- In rats, offspring weights were similar to controls with exposure to Aroclor 1254 and PCB126 (Baker *et al.*, 1977; Meerts *et al.*, 2004; Yang *et al.*, 2009). A decreased offspring weight was observed with exposure to 10 mg/kg-d of Aroclor 1254 (Brezner *et al.*, 1984). An additional study reported changes in rat offspring body weight with exposure to Aroclor 1254 with doses of 1 and 6 mg/kg-d (Yang *et al.*, 2009). Most of the studies in rats examined exposure to a single dose of PCBs, thus no dose-response relationship can be determined.
- In mice, exposed to Aroclor 1242, Aroclor 1254, a mixture of PCB101 and PCB118, PCB126 alone, PCB153 alone, or a mixture of PCB126 and PCB153, no effects were reported on offspring body weight. Doses ranged from 1.2 to 292 mg/kg-d of Aroclors (Fielden *et al.*, 2001; Haake *et al.*, 1987;

Talcott and Koller, 1983) and 0.001 to 750 mg/kg-d for the individual congeners or mixtures of two congeners (Biegel *et al.*, 1989; Mayura *et al.*, 1993; Pocar *et al.*, 2012).

An increase in mice with cleft palates was also reported with gestational doses of 0.52 mg/kg-d PCB126 (Mayura *et al.*, 1993; Zhao *et al.*, 1997) and hydronephrosis [kidney swelling due to inability to excrete urine] with 0.13 mg/kg-d PCB126 or 271 mg/kg-d PCB153 (Biegel *et al.*, 1989).

The study by Pocar *et al.* (2012) also provided information on multigenerational effects. Female mice were exposed to a 50:50 mixture of PCB101 and PCB118 and mated to untreated males. No maternal reproductive or systemic effects were reported. F1 generation female offspring were mated to untreated males. In this generation, litters were smaller in all dose groups by approximately 2 pups. F2 generation female offspring were also mated to untreated males to produce F3. Other than the changes in F1 litter sizes, no other effects on the ability of the F1 through F3 generations to produce viable and reproductively successful offspring were reported. In addition, three generation studies were conducted in rats by Monsanto using Aroclor 1242, Aroclor 1254, and Aroclor 1260 (Industrial Bio-test Laboratories, 1971g, h, i). These studies continued exposure through all three generations, thus it is not possible to separate out effects due to a direct exposure versus a gestational exposure. In these studies, any effects in offspring also occurred with maternal toxicity.

In rat teratogenicity studies using Aroclor 1242, Aroclor 1254, and Aroclor 1260, no increases in teratogenic effects that differed from controls were observed (Industrial Bio-test Laboratories, 1971d, e, f).

4. Female developmental effects

Data on female developmental effects include changes in organ weights, hormonal changes, variations in pubescence, and reductions in fertility in adulthood. I identified 12 publications that met my literature search criteria that assessed female offspring development: one in mice and 11 in rats. I identified only one study—the Litton Bionetics study (Litton Bionetics, 1981)—that reported developmental effects in NHPs; however, this study also reported general toxicity (e.g., swelling of eyes, scaly skin) and maternal toxicity at the same or lower doses. Thus, these findings do not constitute developmental toxicity.

I note variations in female developmental effects based on species, exposure (congener or mixture), and exposure source (gestational or lactational). As shown in Table A6-5, the female offspring developmental effect that is observed to occur at the lowest dose is decreases in ovarian weights in mice (Pocar *et al.*, 2012). However, ovarian weights were unchanged in rats (Gellert and Wilson, 1979; Kobayashi *et al.*, 2009; Meerts *et al.*, 2004; Sager and Girard, 1994; Yang *et al.*, 2009). Conversely, uterine weights were decreased in rats with lactational exposure, but not in either rats or mice with gestational exposure. No effects were reported on pregnancy success in either rats or mice. The weight of evidence suggests no consistent effect on the development of female offspring. Thus, derivation of a POD is not justified using the available data.

These data show:

- In rats, no change in ovarian weights or histopathology was reported with exposure to Aroclor 1254, Aroclor 1221, Aroclor 1242, Aroclor 1260, PCB169, and PCB153. Exposures ranged from 1 mg/kg-d to 30 mg/kg-d and were mostly single dose studies (Gellert and Wilson, 1979; Kobayashi *et al.*, 2009; Meerts *et al.*, 2004; Yang *et al.*, 2009). No effects were reported on ovarian weights with gestational doses of 6-30 mg/kg-d Aroclor 1254 (Gellert and Wilson, 1979; Meerts *et al.*, 2004; Sager and Girard, 1994; Yang *et al.*, 2009). However, effects on rat offspring uterine weights were reported with

lactational exposure to 8 and 32 mg/kg-d Aroclor 1254 (Gellert and Wilson, 1979; Sager and Girard, 1994).

- Although only reported in one study, a decrease in ovarian weight with an increase in atretic follicles was reported in mice with exposure gestationally to 0.001 mg/kg-d (mixture of 101 and PCB118), but not uterine weights up to 0.1 mg/kg-d (Pocar *et al.*, 2012). The lower ovarian weights were significantly different in the F1 generation only; the effects reported in Pocar *et al.* (2012) were significant at higher doses, but no dose-dependent increase in severity was seen.

Development of reproductive organs and accessory organs includes measurement of anogenital distance (AGD), hypospadias, and vaginal development. These effects were only reported in rats; however, a consistent response was seen using several congeners. These studies show:

- In rats, no change in AGD was reported with exposure to 4 mg/kg-d of PCB153 (Kobayashi *et al.*, 2009). Changes were also reported in vaginal development and hypospadias with exposure to 1.8 to 32 mg/kg-d of Aroclor 1254 (Brezner *et al.*, 1984; Meerts *et al.*, 2004; Sager and Girard, 1994).
- In pubescent rats, gestational exposure to 6 mg/kg-d of Aroclor 1254 resulted in delayed vaginal opening (Yang *et al.*, 2009). Lactational exposure resulted in a delay in vaginal opening with 18 mg/kg-d of Aroclor 1254 (Sager and Girard, 1994). Also with lactational exposure, age at first estrous was increased in rats with 18 mg/kg-d of Aroclor 1254, but not 4.4 mg/kg-d (Sager and Girard, 1994). Gestational exposures to 10 mg/kg-d of Aroclor 1254 (only dose tested) was also reported to delay first estrous (Brezner *et al.*, 1984).

Variations in estrous cycle were only reported in studies in rats. Due to the inconsistency in response and the lack of available information presented in the few studies in one species that evaluated estrous cycle, it is not justified to base a POD on this data. These studies show:

- An increase in the number of rats with persistent vaginal estrus, extended estrous, or irregular estrus cycles was reported with lactational exposure to 8 mg/kg-d and greater of Aroclor 1254 (Sager and Girard, 1994), but gestational exposures to 25 mg/kg-d of Aroclor 1254 or 30 mg/kg-d of Aroclor 1212, Aroclor 1242, or Aroclor 1260 had no effect on estrous cycle (Gellert and Wilson, 1979). No differences were reported in estrogen or progesterone with exposures to 25 mg/kg-d of Aroclor 1254 (only dose tested; Meerts *et al.*, 2004)

Mating parameters including implantations and full-term pregnancies were evaluated in both rats and mice; there were no changes reported with gestational exposure. These data are summarized in Table A6-5. These studies show:

- In adult females with either gestational or lactational exposure, no change to mating parameters was reported with 10 mg/kg-d Aroclor 1254 in rats (Brezner *et al.*, 1984), and no increase in anovulatory rats with 30 mg/kg-d Aroclor 1212, Aroclor 1242, or Aroclor 1260 (Gellert and Wilson, 1979). Lactational exposure to 17 mg/kg-d and 36 mg/kg-d caused a decrease in number of embryos and implantations, respectively (Sager and Girard, 1994). No decrease in live births was reported with lactational exposure up to 36 mg/kg-d Aroclor 1254 (Sager and Girard, 1994) or gestational exposure up to 75 mg/kg-d Aroclor 1254 (Brezner *et al.*, 1984).

- In mice, no change in pregnancies that reached term was reported with exposure to a PCB101 and PCB1118 mixture (Pocar *et al.*, 2012).

A clear difference in effect between lactational and gestational exposures as presented in rats was reported; no similar experiments were conducted in NHPs or mice. Effects on uterine weights, pubescent effects, irregular estrous, and implantations were reported with lactational exposure, but either did not occur or occurred at higher doses with gestational exposure to Aroclor 1254 (Sager and Girard, 1994). There is only one study that reports on lactation only effects, thus it is difficult to know the replicability of the results of this study.

Table A6-5. Selected* developmental effects in female offspring and dose ranges of PCBs (mg/kg-d)

Critical Effect	Species	Exposure	Congener	Lowest Dose Tested	NOAEL Range	LOAEL Range	Highest Dose Tested	References
Ovarian weights	Rat	Gestation	Aroclor 1221, Aroclor 1254, Aroclor 1260, PCB153	1	4-30	NA	30	Gellert and Wilson (1979); Kobayashi <i>et al.</i> (2009); Meerts <i>et al.</i> (2004); Yang <i>et al.</i> (2009)
	Mouse	Gestation	PCB101/PCB118 mixture	0.001	NA	0.001	0.1	Pocar <i>et al.</i> (2012)
Uterine weights	Rat	Gestation	Aroclor 1221, Aroclor 1254, Aroclor 1260, PCB153	1	6-30	NA	30	Gellert and Wilson (1979); Meerts <i>et al.</i> (2004); Yang <i>et al.</i> (2009)
	Mouse	Gestation	PCB101/PCB118 mixture	0.001	0.1	NA	0.1	Pocar <i>et al.</i> (2012)
	Rat	Lactation	Aroclor 1254	4.4	4.4	4.4-36	36	Sager and Girard (1994)
Conception	Rat	Lactation	Aroclor 1254	4.4	4.4-36	18-36	36	Sager and Girard (1994)
Pregnancy success	Rat	Gestation	Aroclor 1254	10	10-75	NA	75	Brezner <i>et al.</i> (1984)

Critical Effect	Species	Exposure	Congener	Lowest Dose Tested	NOAEL Range	LOAEL Range	Highest Dose Tested	References
		Lactation	Aroclor 1254	4.4	4.4-36	NA	36	Sager and Girard (1994)
	Mouse	Gestation	PCB101/PCB118 mixture	0.001	0.1	NA	0.1	Pocar <i>et al.</i> (2012)

NA: No values reported in the literature

* Summarizes information only on effects that are potential developmental effects due to consistency across species, reproducibility, and sufficient information on dose-response. Other effects may have been reported in studies, but if not replicated in other studies or consistent with other literature, were not included.

5. Male developmental effects

Developmental effects on male offspring are reported as organ weights, hormonal changes, variations in pubescence, and reductions in sperm quality in adult offspring. I identified four studies in mice and 12 studies in rats that met my literature search criteria and addressed developmental effects of PCBs in male offspring. I identified no studies that reported these effects in NHPs.

Variations in response between species and for different PCBs (congener or mixture) are apparent, as summarized in Table A6-6. However, the most sensitive endpoint for developmental effects on male offspring appears to be changes in testosterone levels. Decreases in testosterone were reported in both rats and mice, although the only study I identified in mice included just one dose. Rat studies report decreases in testosterone with either gestational or lactational exposure and with different congeners. The lowest doses reported to cause a change in this parameter are 0.03 mg/kg-d (PCB169) in rats (Yamamoto *et al.*, 2005) and 0.001 mg/kg-d (PCB101+PCB118) in mice (Fiandanese *et al.*, 2016). Both of these studies included only one dose. For studies with multiple doses tested, the LOAEL for decreases in testosterone in male offspring was 4 mg/kg-d with a mixture of 14 PCBs (Kaya *et al.*, 2002). The NOAEL in this study was 2 mg/kg-d.

Reproductive organ weight changes in male offspring were reported in studies in rats and in mice. Due to the inconsistency in direction of response and effect magnitude, change in organ weight is not a sound endpoint to provide the basis of a POD. These studies show:

- Rat organ weight changes with PCB exposure were inconsistent. In rats exposed to Aroclor 1254, a decrease in prostate, testes, seminal vesicle, and epididymal weights was reported with LOAELs of 1-25 mg/kg-d (Gray *et al.*, 1993; Sager, 1983; Sugantha Priya *et al.*, 2017). However, testes and epididymal weights were increased with exposure to Aroclor 1254 with doses of 21.3 mg/kg-d (Sager, 1983). With 30 mg/kg-d of Aroclor 1260, a decrease in testes weight, but not prostate weight, was reported; the same study found no difference from controls in either testes or prostate weights with the same dose of Aroclor 1221 or Aroclor 1242 (Gellert and Wilson, 1979).
- In mice, with a mixture of PCB101 and PCB118 at 0.001 mg/kg-d, a decrease in testis weight, reduced seminiferous tubule diameter, and disturbances of the relative occurrence of different stages of tubule cycle was reported (Fiandanese *et al.*, 2016; Pocar *et al.*, 2012). These effects on testes weights were statistically significant in the F1 and F2 generations only; however, no increase in severity was seen with dose. No change in testes weights was observed with exposure to 50 mg/kg-d

Aroclor 1242 (Fielden *et al.*, 2001), but a change in prostate weight was reported with 0.05 mg/kg-d Aroclor 1016 (Gupta, 2000).

Similar to studies in female offspring, AGD was evaluated in male offspring. These studies show:

- No effect of Aroclor 1254 or PCB153, on AGD in male rat offspring was reported (Kobayashi *et al.*, 2009; Meerts *et al.*, 2004; Yang *et al.*, 2009) with the exception of one exposure to PCB118 which increased AGD at PND15, but not PND3 (Kuriyama and Chahoud, 2004).
- With mice, AGD was decreased with exposure to 0.001 mg/kg-d of a mixture of PCB101 and PCB118 in the F1 generation only (Pocar *et al.*, 2012) and with exposure to 0.05 mg/kg-d Aroclor 1016 (Gupta, 2000).

Variations in the hormones estradiol and testosterone were evaluated in male rodent offspring. Although the response was inconsistent, this endpoint is summarized in Table A6-6. These studies show:

- In male rat offspring, estradiol was decreased with exposure to 1 mg/kg-d of Aroclor 1254 (Sugantha Priya *et al.*, 2017) but testosterone was unchanged with 25 mg/kg-d (Gray *et al.*, 1993). FSH, LH, and testosterone were all decreased with exposure to 0.03 mg/kg-d PCB169 (Yamamoto *et al.*, 2005), but not PCB126 or PCB169 (Sugantha Priya *et al.*, 2017). A decrease in testosterone was also reported with exposure to a mixture of PCB28, PCB77, PCB101, PCB105, PCB118, PCB126, PCB138, PCB146, PCB153, PCB156, PCB169, PCB170, PCB180, PCB187 or a mixture of PCB28, PCB77, PCB101, PCB118, PCB126 (Kaya *et al.*, 2002; Sugantha Priya *et al.*, 2017). Lactational exposure to 1 mg/kg-d Aroclor 1254 in rats was also reported to reduce testosterone levels (Sugantha Priya *et al.*, 2017).
- Only one study in mice reported testosterone levels, which were said to decrease relative to controls with exposure to 0.001 mg/kg-d PCB101 and PCB118 (only does tested; Fiandanese *et al.*, 2016).

Sperm quality measures were reported in both rats and mice. These effects are summarized in Table A6-6. These studies show:

- Sperm count was decreased in the caudal epididymis in rats, but not testicular or total sperm count (Yang *et al.*, 2009).
- Mice exposed to 0.001 mg/kg-d of a mixture of PCB101 and PCB118 had a decrease in sperm viability, but not sperm production in the F1, F2, and F3 generations, but no increase in severity was seen with increased dose (Fiandanese *et al.*, 2016; Pocar *et al.*, 2012). No changes in sperm quality were seen with exposure to up to 50 mg/kg-d (Fielden *et al.*, 2001). There was no decrease in male rats siring with exposure to 30 mg/kg-d of Aroclor 1221, Aroclor 1242, or Aroclor 1260 (Gellert and Wilson, 1979; Wolf *et al.*, 1999).

Table A6-6. Selected* developmental effects in male offspring and dose ranges of PCBs (mg/kg-d)

Critical Effect	Species	Exposure	Congener	Lowest Dose Tested	NOAEL Range	LOAEL Range	Highest Dose Tested	References
Hormone level	Rat	Gestation	PCB126, PCB169, mixture of 14 [†]	0.003	0.003-2	0.03-4	4	Kaya <i>et al.</i> (2002); Yamamoto <i>et al.</i> (2005)
	Rat	Gestation	Aroclor 1254	0.1	6-25	NA	25	Gray <i>et al.</i> (1993); Yang <i>et al.</i> (2009)
	Rat	Lactation	Aroclor 1254	1	NA	1	5	Sugantha Priya <i>et al.</i> (2017)
	Mouse	Gestation	PCB101/PCB118 mixture	0.001	NA	0.001	0.001	Fiandanese <i>et al.</i> (2016)
Sperm quality	Rat	Gestational	Aroclor 1254, PCB132	0.1	6-25	10-25	25	(Hsu <i>et al.</i> , 2007); Yang <i>et al.</i> (2009)
	Mouse	Gestational	PCB101/PCB118 mixture	0.001	NA	0.001	0.1	Pocar <i>et al.</i> (2012)

* Summarizes information only on effects that are potential developmental effects due to consistency across species, reproducibility, and sufficient information on dose-response. Other effects may have been reported in studies, but if not replicated in other studies or consistent with other literature, were not included.

[†] PCB28, PCB77, PCB101, PCB105, PCB118, PCB126, PCB138, PCB146, PCB153, PCB156, PCB169, PCB170, PCB180, PCB187

F. Exposure assessment

The exposure assessment was documented in Appendix 2. All additional information used for the exposure assessment is detailed in Appendix 5. Both a “best case scenario” (mean fish tissue concentrations and mean fish intake rate) and a “reasonable upper bound” scenario (mean fish tissue concentration and 95th %ile fish intake rate) were used to compare to toxicity values.

G. Risk characterization

In this section I integrate the data from the hazard, dose-response, and exposure assessments above to assess the risk of PCB exposure to people who catch and consume fish from the Spokane River. To do so, I use a Margin of Exposure (MOE) approach, whereby the NOAEL or LOAEL for a given neurodevelopmental test in animals (adjusted to a human-equivalent dose (HED) according to body size scaling factors³⁹ per US EPA) is compared to the estimated PCB intake rate in for those who consume fish from the Spokane River. The general formula to calculate the MOE (adapted for the purposes of this assessment) for a given PCB congener or mixture is as follows:

³⁹ Scaling factors for HED calculations are species-specific as follows: NHP = 1.7, mouse = 7.5 and rat = 4.2 (US EPA, 2011b).

$$MOE = \frac{NOAEL \text{ or } LOAEL \text{ (animal test)} \left(\frac{mg}{kg-d} \right) \times \frac{1}{HED \text{ scaling factor}}}{PCB \text{ consumption rate} \left(\frac{mg}{kg-d} \right)}$$

MOEs represent the “established safety buffer between the toxicity effect dose level and the predicted exposure dose” (US EPA, 2012b). The lower the MOE, the more likely a chemical is to pose an unreasonable risk; for a chemical with an MOE<1, the exposure dose or concentration is greater than the toxicity level. Evaluation of MOEs accounts for standard uncertainty factors used in risk assessment (*e.g.*, interspecies and intraspecies variation, LOAEL to NOAEL), where each uncertainty factor is assigned a value of 10. According to US EPA, an acceptable MOE for LOAEL-based assessments is 1,000 (US EPA, 2012b). For a NOAEL-based assessment, an acceptable MOE would be 100.

1. Reproductive effects

For both the “best case scenario” and the “reasonable upper bound” scenario, I identified no reproductive studies with NOAEL-based MOEs below 100 and two with LOAEL-based MOEs less than 1000: Arnold *et al.* (1995) and Truelove *et al.* (1990). There was no NOAEL reported for this effect in Truelove *et al.* (1990). These studies are summarized in Table A6-7.

Table A6-7. Reproductive studies with LOAEL-based MOEs less than 1,000

Study	Species	Congener and Dose(s)	Lifestage	Reported Effects	“best case scenario”		“reasonable upper bound”	
					NOAEL MOE	LOAEL MOE	NOAEL MOE	LOAEL MOE
Arnold <i>et al.</i> (1995)	NHP	Aroclor 1254 0, 0.005, 0.02, 0.040 or 0.080 mg/kg-d	Maternal	Decreased conception with 0.02 mg/kg-d	929	>1,000	237	949
Truelove <i>et al.</i> (1990)	NHP	Aroclor 1254 0, 0.005, 0.02, 0.040 or 0.080 mg/kg-d	Maternal	Non-dose-dependent reduction in progesterone at 0.005 mg/kg-d (equivocal finding per authors)	NA	929	NA	237

Arnold *et al.* (1995) and Truelove *et al.* (1990) are not independent studies and represent different publications on the same cohort of animals. For both “best case” and “reasonable upper bound” exposure scenarios, a LOAEL was reported for Arnold *et al.* (1995) that was less than 1,000, and Truelove *et al.* (1990) reported only a LOAEL (*i.e.*, there was no NOAEL), which corresponded to an MOE less than 1,000. Arnold *et al.* (1995) reported that conception was decreased in the 0.02, 0.04, or 0.08 mg/kg-d dose groups. Although the reduction in progesterone reported in the lowest dose tested in Truelove *et al.* (1990) was statistically significant and the basis of the LOAEL, it was not considered biologically significant by the study authors. The

authors noted, “Since the statistically significant changes found in progesterone concentration did not occur in a dose related manner (the 20 and 40 µg/kg-d dose groups and all of test group 2 showed no differences) the toxicologic significance of this observation is equivocal.”

2. Developmental effects

For the “best case scenario,” I identified two developmental studies with LOAELs corresponding to MOEs less than 1,000: Fiandanese *et al.* (2016) and Pocar *et al.* (2012). There were no NOAEL-based MOEs less than 100.

For the “reasonable upper bound” scenario, both Fiandanese *et al.* (2016) and Pocar *et al.* (2012) had LOAEL-based MOEs less than 1,000, as did Gupta (2000). There were no NOAEL-based MOEs less than 100. These are summarized in Table A6-8.

Table A6-8. Developmental studies with LOAEL-based MOEs less than 1,000

Study	Species	Congener and Dose(s)	Lifestage	Reported Effect	"best case scenario"		"reasonable upper bound"	
					NOAEL MOE	LOAEL MOE	NOAEL MOE	LOAEL MOE
Fiandanese <i>et al.</i> (2016)	CD-1 mice	1:1 of PCB101 and 118 0 or 0.001 mg/kg-d (only dose tested)	Male offspring	Decrease in testis weight, reduced seminiferous tubule diameter, and disturbances of the relative occurrence of different stages of tubule cycles. There was also a decrease in sperm viability, but not sperm production.	NA	337	NA	86
Gupta (2000)	CD-1 mice	Aroclor 1016 0 or 0.050 mg/kg-d (only dose tested)	Male offspring	Increased AGD, prostate weight, and decreased epididymal weight, increased AR binding	NA	>1,000	NA	308
Pocar <i>et al.</i> (2012)	CD-1 mice	1:1 of PCB101 and 118 0, 0.001, 0.01 or 0.1 mg/kg-d	Male F1, F2, F3; Female F1	Decreased testes weight, tubule diameter, sperm viability at 0.001 mg/kg-d for F1/2. Decrease in sperm viability also at F3 0.001 mg/kg-d. Decrease in ovary weight in F1 at 0.001 mg/kg-d.	NA	337	NA	86*

*The next highest dose (0.01 mg/kg-d) also corresponds to an MOE below 1,000.

Based on my review, I have found that these three studies with LOAEL-based MOEs less than 1,000 are not sufficiently reliable to provide the basis for a POD for developmental effects of PCBs, particularly at the environmental exposure levels associated with intake of PCBs from fish in the Spokane River.

First, Pocar *et al.* (2012) and Fiandanese *et al.* (2016) are publications from the same laboratory and use the same mouse model and same exposure protocol with exposure to the same mixture of 50:50 PCB101 and PCB118. I did not identify any other laboratory that has studied this mixture, and its relevance to human exposure is unclear since a 50:50 mixture is not commonly reported in fish.

Further, the results from these studies are not consistent with other literature. As a result, it is difficult to compare the results of these studies to others to determine whether they are reliable. For example, Pocar *et al.* (2012) report that testes weights were decreased with maternal exposure to the PCB101/118 mixture at doses above 0.001 mg/kg-d, but other studies examining other congeners and in other species do not report a change in this endpoint (Gellert and Wilson, 1979). Across all congeners, the range of LOAELs for decrease in testes weights in offspring is 0.001-50 mg/kg-d. However, the range of NOAELs overlaps the LOAEL range and ranges as high as 64 mg/kg-d. The next lowest LOAEL for this endpoint is 0.17 mg/kg-d (for A1254), two orders of magnitude greater than the LOAEL reported in Pocar *et al.* (2012) for the PCB101/118 mixture. Consistency and replicability in response are key in elucidating a real from a spurious effect.

Second, Pocar *et al.* (2012) presents data on three generations of mice following maternal P1 administration of one of four dose groups. While some of the reported effects in the F1, F2, and F3 generations were significantly different from controls, no dose-dependent increase in severity was observed for any of the endpoints. For example, testes weights are significantly decreased at the lowest dose group, but the weights do not continue to decrease with increasing dose. This suggests that there may have been a systematic difference in how treated animals were handled relative to controls, leading to a difference in all exposed animals that was independent of dose. With no other studies evaluating exposure of three generations to this unique mixture, the relevance of these findings to human populations is unclear.

Finally, the studies by Gupta (2000) and Fiandanese *et al.* (2016) only tested one dose (of Aroclor 1016 or the mixture of PCB101 and PCB118, respectively). Although significant results are presented, studies with only one dose do not allow for an assessment of dose-response under the conditions of the study or a clear assessment of whether the effects are even treatment related.

Based on these limitations, these three studies do not independently suggest that developmental effects could occur with environmental exposures to PCBs from fish from the Spokane River.

While I estimate several MOEs for reproductive and developmental endpoints that are less than 1,000, several considerations suggest that there is not a significant risk to people who consume fish caught in the Spokane River. First, although the LOAEL from Pocar *et al.* (2012) yields an MOE of 86, this value indicates that exposure, even to maximally exposed individuals, is below the doses evaluated in Pocar *et al.* (2012) or Fiandanese *et al.* (2016), which I concluded are not reliable for determining a POD as described above. Second, the MOEs are calculated using an extremely conservative upper bound estimate of exposure that is based on the sum of 95th percentile fish consumption rates for individual species (see Exposure Assessment). This likely greatly overestimates the amount of fish any single person would consume repeatedly on a daily

basis. Finally, in this scenario, a female would have to eat this amount of fish daily prior to and during pregnancy for there to be a potential for adverse developmental effects.

The assessment I conducted makes both “best case” and “reasonable upper bound” assumptions about the amount of fish consumed from the Spokane River. Using conservative assumptions about exposure is one (of many) ways a toxicologist can ensure that risks estimated in a human health risk assessment are not underestimated. In this assessment, I assume that every day, a person consumes each species of fish caught from the Spokane River at a rate that is either average or near the maximum consumption rate for all people who catch and consume this fish (i.e., at the mean and 95th percentile of the consumption rate for each species of fish). The “reasonable upper bound” is not a plausible scenario; however, I use this conservative consumption rate in my assessment to calculate the margins of exposure (MOEs) for each study I identify as relevant. Even estimates representing a “best case scenario” are unlikely given the amount and types of fish necessary for consumption on a daily basis from the Spokane River. Thus, the reader should consider the level of conservatism when assessing the results of this toxicological assessment.

Based on this assessment of the hazard, exposure, and toxicity of PCBs, I conclude that it is unlikely that any reproductive or developmental effects will occur from exposure to PCBs in fish caught and consumed from the Spokane River.

XI. References

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XII. Attachments

A. Attachment 1. Monsanto studies and bulletins

1. Attachment 1A. List of PCB-related toxicology studies conducted under contract from Monsanto

Item	Year	Study design	Species - Test article	Exposure route	Bates Number
1	1934	Aroclor 1248 (lot 5)	Rabbit - Dermal	Irritation	[TOXSTUDIES0001]
2	1934	Aroclor 1248 (lot 9)	Rabbit - Dermal	Irritation	[TOXSTUDIES0001]
3	1934	Aroclor 1248 Special (batch 8)	Rabbit - Dermal	Irritation	[TOXSTUDIES0001]
4	1934	Aroclor 1260 (lot 32)	Rabbit - Dermal	Irritation	[TOXSTUDIES0001]
5	1934	Aroclor 1262 (batch 8a)	Rabbit - Dermal	Irritation	[TOXSTUDIES0001]
6	1934	Aroclor 1262 (lot 3)	Rabbit - Dermal	Irritation	[TOXSTUDIES0001]
7	1934	Aroclor 1262 (lot notebook 176, p.121)	Rabbit - Dermal	Irritation	[TOXSTUDIES0001]
8	1934	Aroclor 1269 (lot 3, dry ground)	Rabbit - Dermal	Irritation	[TOXSTUDIES0001]
9	1934	Aroclor 1269 (lot 3, notebook 192, p.10, dry ground in pebble mill)	Rabbit - Dermal	Irritation	[TOXSTUDIES0001]
10	1934	Aroclor 1269 (lot notebook 176, p.121, dry ground)	Rabbit - Dermal	Irritation	[TOXSTUDIES0001]
11	1934	Aroclor 1269 (lot notebook 176, p.121; wet ground)	Rabbit - Dermal	Irritation	[TOXSTUDIES0001]
12	1934	Aroclor Special (lot notebook 192, p.9)	Rabbit - Dermal	Irritation	[TOXSTUDIES0001]
13	1934	Halowax 1000	Rabbit - Dermal	Irritation	[TOXSTUDIES0001]
14	1934	Halowax 1001	Rabbit - Dermal	Irritation	[TOXSTUDIES0001]
15	1934	Halowax 1004	Rabbit - Dermal	Irritation	[TOXSTUDIES0001]
16	1937	Chlorinated diphenyl (65% chlorine)	Rat - Inhalation	Repeated dose	[TOXSTUDIES0005]
17	1937	Chlorinated diphenyl (65% chlorine)	Rat - Inhalation	Repeated dose	[TOXSTUDIES0005]
18	1937	Chlorinated naphthalenes + chlorinated diphenyl	Rat - Inhalation	Repeated dose	[TOXSTUDIES0005]
19	1937	Chlorinated naphthalenes + chlorinated diphenyl	Rat - Inhalation	Repeated dose	[TOXSTUDIES0005]
20	1937	Chlorinated diphenyl (65% chlorine)	Rat - Oral	Repeated dose	[TOXSTUDIES0005]
21	1937	Chlorinated naphthalenes + chlorinated diphenyl	Rat - Oral	Repeated dose	[TOXSTUDIES0005]
22	1938	Aroclor 1268	Rat - Inhalation	Repeated dose	[TOXSTUDIES00061]

Item	Year	Study design	Species - Test article	Exposure route	Bates Number
23	1938	Aroclor 4465	Rat - Inhalation	Repeated dose	[TOXSTUDIES0074]
24	1938	Aroclor 4465	Rat - Inhalation	Repeated dose	[TOXSTUDIES0074]
25	1938	Aroclor 5460	Rat - Inhalation	Repeated dose	[TOXSTUDIES0061]
26	1938	Chlorinated diphenyl (65% chlorine)	Rat - Inhalation	Repeated dose	[TOXSTUDIES0034]
27	1938	Chlorinated diphenyl (65% chlorine)	Rat - Inhalation	Repeated dose	[TOXSTUDIES0034]
28	1938	Chlorinated naphthalenes + chlorinated diphenyl	Rat - Inhalation	Repeated dose	[TOXSTUDIES0034]
29	1938	Chlorinated naphthalenes + chlorinated diphenyl	Rat - Inhalation	Repeated dose	[TOXSTUDIES0034]
30	1938	Chlorinated diphenyl (65% chlorine)	Rat - Oral	Repeated dose	[TOXSTUDIES0034]
31	1938	Chlorinated naphthalenes + chlorinated diphenyl	Rat - Oral	Repeated dose	[TOXSTUDIES0034]
32	1938	Chlorinated naphthalenes + chlorinated diphenyl	Rat - Oral	Repeated dose	[TOXSTUDIES0034]
33	1949	Aroclor [# not specified]	Human - Dermal	Irritation	[TOXSTUDIES0092]
34	1951	Pydraul F-9	Human - Dermal	Irritation	[TOXSTUDIES0098]
35	1951	Pydraul F-9	Rabbit - Oral	MLD	[TOXSTUDIES0094]
36	1951	Pydraul F-9	Rat - Oral	MLD	[TOXSTUDIES0096]
37	1953	Aroclor 1248	Multiple (cat, guinea pig, rabbit, rat) - Inhalation	Repeated dose	[TOXSTUDIES0100]
38	1953	Pydraul F-9	Multiple (cat, guinea pig, rabbit, rat) - Inhalation	Repeated dose	[TOXSTUDIES0100]
39	1953	Aroclor 1248	Rabbit - Dermal	Acute (single exposure)	[TOXSTUDIES0100]
40	1953	Pydraul F-9	Rabbit - Dermal	Acute (single exposure)	[TOXSTUDIES0100]
41	1953	Aroclor 1242	Rat - Oral	LD50	[TOXSTUDIES0185]
42	1953	Aroclor 1254	Rat - Oral	LD50	[TOXSTUDIES0182]
43	1954	OS-57	Rabbit - Dermal	MLD	[TOXSTUDIES0194]
44	1954	OS-57	Rabbit - Dermal	Irritation	[TOXSTUDIES0194]
45	1954	OS-54	Rabbit - Inhalation	Repeated dose	[TOXSTUDIES0188]
46	1954	OS-54	Rabbit - Ocular	Irritation	[TOXSTUDIES0188]
47	1954	OS-57	Rabbit - Ocular	Irritation	[TOXSTUDIES0194]
48	1954	OS-57	Rat - Inhalation	Acute (single exposure)	[TOXSTUDIES0194]
49	1954	OS-54	Rat - Oral	LD50	[TOXSTUDIES0188]
50	1954	OS-57	Rat - Oral	Acute (single exposure)	[TOXSTUDIES0194]

Item	Year	Study design	Species - Test article	Exposure route	Bates Number
51	1955	Aroclor 1242	Multiple (cat, guinea pig, rabbit, rat) - Inhalation	Repeated dose	[TOXSTUDIES0212]
52	1955	Aroclor 1242	Multiple (cat, guinea pig, rabbit, rat) - Inhalation	Repeated dose	[TOXSTUDIES0212]
53	1955	Aroclor 1242	Multiple (cat, guinea pig, rabbit, rat) - Inhalation	Repeated dose	[TOXSTUDIES0314]
54	1955	Aroclor 1254	Multiple (cat, guinea pig, rabbit, rat) - Inhalation	Repeated dose	[TOXSTUDIES0212]
55	1955	Aroclor 1254	Multiple (cat, guinea pig, rabbit, rat) - Inhalation	Repeated dose	[TOXSTUDIES0314]
56	1955	Pydraul F-9	Multiple (cat, guinea pig, rabbit, rat) - Inhalation	Acute (single exposure)	[TOXSTUDIES0388]
57	1955	Pydraul F-9	Multiple (cat, guinea pig, rabbit, rat) - Inhalation	Acute (single exposure)	[TOXSTUDIES0388]
58	1955	OS-63	Rabbit - Dermal	MLD	[TOXSTUDIES0206]
59	1955	OS-63	Rabbit - Dermal	Irritation	[TOXSTUDIES0206]
60	1955	Pydraul 600	Rabbit - Dermal	MLD	[TOXSTUDIES0404]
61	1955	Pydraul 600	Rabbit - Dermal	Irritation	[TOXSTUDIES0404]
62	1955	Pydraul F-9	Rabbit - Dermal	MLD	[TOXSTUDIES0200]
63	1955	Pydraul F-9	Rabbit - Dermal	Irritation	[TOXSTUDIES0200]
64	1955	OS-63	Rabbit - Ocular	Irritation	[TOXSTUDIES0206]
65	1955	Pydraul 600	Rabbit - Ocular	Irritation	[TOXSTUDIES0404]
66	1955	Pydraul F-9	Rabbit - Ocular	Irritation	[TOXSTUDIES0200]
67	1955	Pydraul 600	Rabbit - Oral	LD50	[TOXSTUDIES0410]
68	1955	OS-63	Rat - Inhalation	Acute (single exposure)	[TOXSTUDIES0206]
69	1955	Pydraul 600	Rat - Inhalation	Acute (single exposure)	[TOXSTUDIES0404]
70	1955	Pydraul F-9	Rat - Inhalation	Acute (single exposure)	[TOXSTUDIES0200]
71	1955	OS-63	Rat - Oral	LD50	[TOXSTUDIES0206]
72	1955	Pydraul 600	Rat - Oral	LD50	[TOXSTUDIES0404]
73	1955	Pydraul 600	Rat - Oral	Acute (single exposure)	[TOXSTUDIES0410]
74	1955	Pydraul F-9	Rat - Oral	LD50	[TOXSTUDIES0200]
75	1956	Pydraul AC	Rabbit - Dermal	MLD	[TOXSTUDIES0413]
76	1956	Pydraul AC	Rabbit - Dermal	Irritation	[TOXSTUDIES0413]
77	1956	Pydraul AC	Rabbit - Ocular	Irritation	[TOXSTUDIES0413]
78	1956	Pydraul AC	Rabbit - Oral	MLD	[TOXSTUDIES0413]
79	1956	Pydraul AC	Rat - Inhalation	Acute (single exposure)	[TOXSTUDIES0413]
80	1956	Pydraul AC	Rat - Oral	LD50	[TOXSTUDIES0413]

Item	Year	Study design	Species - Test article	Exposure route	Bates Number
81	1957	Aroclor 1270	Rabbit - Dermal	Irritation	[TOXSTUDIES0430]
82	1957	OS-81	Rabbit - Dermal	Irritation	[TOXSTUDIES0420]
83	1957	OS-83	Rabbit - Dermal	Irritation	[TOXSTUDIES0420]
84	1957	Aroclor 1270	Rabbit - Ocular	Irritation	[TOXSTUDIES0430]
85	1957	OS-81	Rabbit - Ocular	Irritation	[TOXSTUDIES0420]
86	1957	OS-83	Rabbit - Ocular	Irritation	[TOXSTUDIES0420]
87	1957	OS-81	Rabbit - Oral	MLD	[TOXSTUDIES0420]
88	1957	OS-83	Rabbit - Oral	MLD	[TOXSTUDIES0420]
89	1957	OS-81	Rat - Inhalation	Acute (single exposure)	[TOXSTUDIES0420]
90	1957	OS-83	Rat - Inhalation	Acute (single exposure)	[TOXSTUDIES0420]
91	1957	Aroclor 1270	Rat - Oral	LD50	[TOXSTUDIES0430]
92	1957	OS-81	Rat - Oral	LD50	[TOXSTUDIES0420]
93	1957	OS-83	Rat - Oral	LD50	[TOXSTUDIES0420]
94	1958	Aroclor 1268	Rabbit - Dermal	MLD	[TOXSTUDIES0448]
95	1958	Aroclor 1268	Rabbit - Dermal	Irritation	[TOXSTUDIES0448]
96	1958	OS-95	Rabbit - Dermal	MLD	[TOXSTUDIES0433]
97	1958	OS-95	Rabbit - Dermal	Irritation	[TOXSTUDIES0433]
98	1958	OS-95	Rabbit - Dermal	MLD	[TOXSTUDIES0463]
99	1958	OS-95	Rabbit - Dermal	MLD	[TOXSTUDIES0463]
100	1958	OS-95	Rabbit - Dermal	Irritation	[TOXSTUDIES0463]
101	1958	OS-95	Rabbit - Dermal	Irritation	[TOXSTUDIES0463]
102	1958	Aroclor 1268	Rabbit - Ocular	Irritation	[TOXSTUDIES0448]
103	1958	OS-95	Rabbit - Ocular	Irritation	[TOXSTUDIES0433]
104	1958	OS-95	Rat - Inhalation	Acute (single exposure)	[TOXSTUDIES0433]
105	1958	OS-95	Rat - Inhalation	Acute (single exposure)	[TOXSTUDIES0467]
106	1958	OS-95	Rat - Inhalation	Repeated dose	[TOXSTUDIES0467]
107	1958	Pydraul 625	Rat - Inhalation	Acute (single exposure)	[TOXSTUDIES0440]
108	1958	Pydraul 625	Rat - Inhalation	Repeated dose	[TOXSTUDIES0440]
109	1958	Aroclor 1268	Rat - Oral	LD50	[TOXSTUDIES0448]
110	1958	OS-95	Rat - Oral	LD50	[TOXSTUDIES0433]
111	1962	Aroclor 1221	Rabbit - Dermal	MLD	[TOXSTUDIES0479]
112	1962	Aroclor 1232	Rabbit - Dermal	MLD	[TOXSTUDIES0475]
113	1962	Aroclor 1242	Rabbit - Dermal	MLD	[TOXSTUDIES0483]
114	1962	Aroclor 1248	Rabbit - Dermal	MLD	[TOXSTUDIES0487]
115	1962	Aroclor 1254	Rabbit - Dermal	MLD	[TOXSTUDIES0495]
116	1962	Aroclor 1260	Rabbit - Dermal	MLD	[TOXSTUDIES0491]
117	1962	Aroclor 1262	Rabbit - Dermal	MLD	[TOXSTUDIES0499]
118	1962	Aroclor 1268	Rabbit - Dermal	MLD	[TOXSTUDIES0509]
119	1962	Aroclor 2565	Rabbit - Dermal	MLD	[TOXSTUDIES0513]
120	1962	Aroclor 4465	Rabbit - Dermal	MLD	[TOXSTUDIES0503]

Item	Year	Study design	Species - Test article	Exposure route	Bates Number
121	1962	Aroclor 4465	Rabbit - Dermal	Irritation	[TOXSTUDIES0503]
122	1962	Aroclor 4465	Rabbit - Ocular	Irritation	[TOXSTUDIES0503]
123	1962	Aroclor 1221	Rat - Oral	LD50	[TOXSTUDIES0479]
124	1962	Aroclor 1232	Rat - Oral	LD50	[TOXSTUDIES0475]
125	1962	Aroclor 1242	Rat - Oral	LD50	[TOXSTUDIES0483]
126	1962	Aroclor 1248	Rat - Oral	LD50	[TOXSTUDIES0487]
127	1962	Aroclor 1254	Rat - Oral	LD50	[TOXSTUDIES0495]
128	1962	Aroclor 1260	Rat - Oral	LD50	[TOXSTUDIES0491]
129	1962	Aroclor 1262	Rat - Oral	LD50	[TOXSTUDIES0499]
130	1962	Aroclor 1268	Rat - Oral	LD50	[TOXSTUDIES0509]
131	1962	Aroclor 2565	Rat - Oral	LD50	[TOXSTUDIES0513]
132	1962	Aroclor 4465	Rat - Oral	LD50	[TOXSTUDIES0503]
133	1963	Aroclor 1221	Rabbit - Dermal	Repeated dose	[TOXSTUDIES0583]
134	1963	Aroclor 1242	Rabbit - Dermal	Repeated dose	[TOXSTUDIES0549]
135	1963	Aroclor 1248	Rabbit - Dermal	Repeated dose	[TOXSTUDIES0533]
136	1963	Aroclor 1254	Rabbit - Dermal	Repeated dose	[TOXSTUDIES0602]
137	1963	Aroclor 1268	Rabbit - Dermal	Repeated dose	[TOXSTUDIES0566]
138	1963	Aroclor 4465	Rabbit - Dermal	Repeated dose	[TOXSTUDIES0620]
139	1963	Inerteen PPO	Rabbit - Dermal	MLD	[TOXSTUDIES0525]
140	1963	Inerteen PPO	Rabbit - Dermal	Irritation	[TOXSTUDIES0525]
141	1963	MCS 300	Rabbit - Dermal	MLD	[TOXSTUDIES0637]
142	1963	MCS 300	Rabbit - Dermal	Irritation	[TOXSTUDIES0637]
143	1963	Pyranol 1470	Rabbit - Dermal	MLD	[TOXSTUDIES0517]
144	1963	Pyranol 1470	Rabbit - Dermal	Irritation	[TOXSTUDIES0517]
145	1963	Inerteen PPO	Rabbit - Ocular	Irritation	[TOXSTUDIES0525]
146	1963	MCS 300	Rabbit - Ocular	Irritation	[TOXSTUDIES0637]
147	1963	Pyranol 1470	Rabbit - Ocular	Irritation	[TOXSTUDIES0517]
148	1963	Inerteen PPO	Rat - Inhalation	Acute (single exposure)	[TOXSTUDIES0525]
149	1963	MCS 300	Rat - Inhalation	Acute (single exposure)	[TOXSTUDIES0637]
150	1963	Pyranol 1470	Rat - Inhalation	Acute (single exposure)	[TOXSTUDIES0517]
151	1963	Inerteen PPO	Rat - Oral	LD50	[TOXSTUDIES0525]
152	1963	MCS 300	Rat - Oral	LD50	[TOXSTUDIES0637]
153	1963	Pyranol 1470	Rat - Oral	LD50	[TOXSTUDIES0517]
154	1964	FH-145	Rabbit - Dermal	MLD	[TOXSTUDIES0645]
155	1964	FH-145	Rabbit - Dermal	Irritation	[TOXSTUDIES0645]
156	1964	FH-145	Rabbit - Dermal	Irritation	[TOXSTUDIES0654]
157	1964	FH-159	Rabbit - Dermal	MLD	[TOXSTUDIES0662]
158	1964	FH-159	Rabbit - Dermal	Irritation	[TOXSTUDIES0662]
159	1964	MCS 295	Rabbit - Dermal	MLD	[TOXSTUDIES0654]
160	1964	Pydraul 280	Rabbit - Dermal	MLD	[TOXSTUDIES0670]
161	1964	Pydraul 280	Rabbit - Dermal	Irritation	[TOXSTUDIES0670]

Item	Year	Study design	Species - Test article	Exposure route	Bates Number
162	1964	Pydraul 312 (MCS 312)	Rabbit - Dermal	Irritation	[TOXSTUDIES0678]
163	1964	Pydraul 312 (MCS 312)	Rabbit - Dermal	MLD	[TOXSTUDIES0678]
164	1964	FH-145	Rabbit - Ocular	Irritation	[TOXSTUDIES0645]
165	1964	FH-145	Rabbit - Ocular	Irritation	[TOXSTUDIES0654]
166	1964	FH-159	Rabbit - Ocular	Irritation	[TOXSTUDIES0662]
167	1964	Pydraul 280	Rabbit - Ocular	Irritation	[TOXSTUDIES0670]
168	1964	Pydraul 312 (MCS 312)	Rabbit - Ocular	Irritation	[TOXSTUDIES0678]
169	1964	FH-145	Rabbit - Oral	MLD	[TOXSTUDIES0645]
170	1964	FH-145	Rat - Inhalation	Acute (single exposure)	[TOXSTUDIES0645]
171	1964	FH-145	Rat - Inhalation	Acute (single exposure)	[TOXSTUDIES0654]
172	1964	FH-159	Rat - Inhalation	Acute (single exposure)	[TOXSTUDIES0662]
173	1964	Pydraul 280	Rat - Inhalation	Acute (single exposure)	[TOXSTUDIES0670]
174	1964	Pydraul 312 (MCS 312)	Rat - Inhalation	Acute (single exposure)	[TOXSTUDIES0678]
175	1964	FH-145	Rat - Oral	LD50	[TOXSTUDIES0645]
176	1964	FH-159	Rat - Oral	LD50	[TOXSTUDIES0662]
177	1964	MCS 295	Rat - Oral	LD50	[TOXSTUDIES0654]
178	1964	Pydraul 280	Rat - Oral	LD50	[TOXSTUDIES0670]
179	1964	Pydraul 312 (MCS 312)	Rat - Oral	LD50	[TOXSTUDIES0678]
180	1966	MCS 153	Rabbit - Dermal	MLD	[TOXSTUDIES0740]
181	1966	MCS 395	Rabbit - Dermal	MLD	[TOXSTUDIES0686]
182	1966	MCS 395	Rabbit - Dermal	MLD	[TOXSTUDIES0686]
183	1966	MCS 395	Rabbit - Dermal	Irritation	[TOXSTUDIES0686]
184	1966	MCS 404	Rabbit - Dermal	MLD	[TOXSTUDIES0695]
185	1966	MCS 404	Rabbit - Dermal	Irritation	[TOXSTUDIES0695]
186	1966	MCS 404	Rabbit - Dermal	MLD	[TOXSTUDIES0732]
187	1966	MCS 528	Rabbit - Dermal	MLD	[TOXSTUDIES0712]
188	1966	MCS 90	Rabbit - Dermal	MLD	[TOXSTUDIES0704]
189	1966	MCS 90	Rabbit - Dermal	Irritation	[TOXSTUDIES0704]
190	1966	Pydraul 135	Rabbit - Dermal	MLD	[TOXSTUDIES0724]
191	1966	Pydraul 280	Rabbit - Dermal	MLD	[TOXSTUDIES0720]
192	1966	Pydraul 625	Rabbit - Dermal	MLD	[TOXSTUDIES0728]
193	1966	Pydraul AC	Rabbit - Dermal	MLD	[TOXSTUDIES0716]
194	1966	Pydraul F-9	Rabbit - Dermal	MLD	[TOXSTUDIES0736]
195	1966	MCS 395	Rabbit - Ocular	Irritation	[TOXSTUDIES0686]
196	1966	MCS 404	Rabbit - Ocular	Irritation	[TOXSTUDIES0695]
197	1966	MCS 90	Rabbit - Ocular	Irritation	[TOXSTUDIES0704]
198	1966	MCS 404	Rabbit - Oral	MLD	[TOXSTUDIES0695]
199	1966	MCS 395	Rat - Inhalation	Acute (single exposure)	[TOXSTUDIES0686]

Item	Year	Study design	Species - Test article	Exposure route	Bates Number
200	1966	MCS 404	Rat - Inhalation	Acute (single exposure)	[TOXSTUDIES0695]
201	1966	MCS 90	Rat - Inhalation	Acute (single exposure)	[TOXSTUDIES0704]
202	1966	MCS 153	Rat - Oral	LD50	[TOXSTUDIES0740]
203	1966	MCS 395	Rat - Oral	LD50	[TOXSTUDIES0686]
204	1966	MCS 404	Rat - Oral	LD50	[TOXSTUDIES0695]
205	1966	MCS 404	Rat - Oral	LD50	[TOXSTUDIES0732]
206	1966	MCS 528	Rat - Oral	LD50	[TOXSTUDIES0712]
207	1966	MCS 90	Rat - Oral	LD50	[TOXSTUDIES0704]
208	1966	Pydraul 135	Rat - Oral	LD50	[TOXSTUDIES0724]
209	1966	Pydraul 280	Rat - Oral	LD50	[TOXSTUDIES0720]
210	1966	Pydraul 625	Rat - Oral	LD50	[TOXSTUDIES0728]
211	1966	Pydraul AC	Rat - Oral	LD50	[TOXSTUDIES0716]
212	1966	Pydraul F-9	Rat - Oral	LD50	[TOXSTUDIES0736]
213	1967	Pydraul 230	Rabbit - Dermal	MLD	[TOXSTUDIES0744]
214	1967	Pydraul 230	Rabbit - Dermal	Irritation	[TOXSTUDIES0744]
215	1967	Pydraul 230	Rabbit - Ocular	Irritation	[TOXSTUDIES0744]
216	1967	Pydraul 230	Rat - Inhalation	Acute (single exposure)	[TOXSTUDIES0744]
217	1967	(XA-140) Santicizer 1706	Rat - Oral	LD50	[TOXSTUDIES0752]
218	1967	Pydraul 230	Rat - Oral	LD50	[TOXSTUDIES0744]
219	1969	Aroclor 1242	Bluegill fish - Water	LC50	[TOXSTUDIES0798]
220	1969	Aroclor 1254	Bluegill fish - Water	LC50	[TOXSTUDIES0798]
221	1969	Aroclor 1260	Bluegill fish - Water	LC50	[TOXSTUDIES0798]
222	1969	Aroclor 5460	Bluegill fish - Water	LC50	[TOXSTUDIES0798]
223	1969	Halowax 1099	Bluegill fish - Water	LC50	[TOXSTUDIES0798]
224	1969	Toxaphene	Bluegill fish - Water	LC50	[TOXSTUDIES0798]
225	1969	Aroclor 1242	Chicken - Oral	LD50	[TOXSTUDIES0773]
226	1969	Aroclor 1242	Chicken - Oral	Tissue burden	[TOXSTUDIES0773]
227	1969	Aroclor 1254	Chicken - Oral	LD50	[TOXSTUDIES0773]
228	1969	Aroclor 1254	Chicken - Oral	Tissue burden	[TOXSTUDIES0773]
229	1969	Aroclor 1260	Chicken - Oral	LD50	[TOXSTUDIES0773]
230	1969	Aroclor 1260	Chicken - Oral	Tissue burden	[TOXSTUDIES0773]
231	1969	Aroclor 5460	Chicken - Oral	LD50	[TOXSTUDIES0773]
232	1969	Aroclor 5460	Chicken - Oral	Tissue burden	[TOXSTUDIES0773]
233	1969	Halowax 1014	Chicken - Oral	LD50	[TOXSTUDIES0773]
234	1969	Halowax 1014	Chicken - Oral	Tissue burden	[TOXSTUDIES0773]
235	1969	Halowax 1099	Chicken - Oral	LD50	[TOXSTUDIES0773]
236	1969	Halowax 1099	Chicken - Oral	Tissue burden	[TOXSTUDIES0773]
237	1969	Toxaphene	Chicken - Oral	LD50	[TOXSTUDIES0773]
238	1969	Toxaphene	Chicken - Oral	Tissue burden	[TOXSTUDIES0773]
239	1969	MCS 528	Rabbit - Dermal	MLD	[TOXSTUDIES0754]

Item	Year	Study design	Species - Test article	Exposure route	Bates Number
240	1969	MCS 528	Rabbit - Dermal	Irritation	[TOXSTUDIES0754]
241	1969	MCS 900	Rabbit - Dermal	MLD	[TOXSTUDIES0789]
242	1969	MCS 900	Rabbit - Dermal	Irritation	[TOXSTUDIES0789]
243	1969	MCS 9001	Rabbit - Dermal	MLD	[TOXSTUDIES0832]
244	1969	MCS 9001	Rabbit - Dermal	Irritation	[TOXSTUDIES0832]
245	1969	Pydraul 312	Rabbit - Dermal	Irritation	[TOXSTUDIES0760]
246	1969	Pydraul 312	Rabbit - Dermal	MLD	[TOXSTUDIES0760]
247	1969	MCS 528	Rabbit - Ocular	Irritation	[TOXSTUDIES0754]
248	1969	MCS 900	Rabbit - Ocular	Irritation	[TOXSTUDIES0789]
249	1969	MCS 9001	Rabbit - Ocular	Irritation	[TOXSTUDIES0832]
250	1969	Pydraul 312	Rabbit - Ocular	Irritation	[TOXSTUDIES0760]
251	1969	Aroclor 1242	Rainbow trout - Water	LC50	[TOXSTUDIES0798]
252	1969	Aroclor 1254	Rainbow trout - Water	LC50	[TOXSTUDIES0798]
253	1969	Aroclor 1260	Rainbow trout - Water	LC50	[TOXSTUDIES0798]
254	1969	Aroclor 5460	Rainbow trout - Water	LC50	[TOXSTUDIES0798]
255	1969	Halowax 1099	Rainbow trout - Water	LC50	[TOXSTUDIES0798]
256	1969	Toxaphene	Rainbow trout - Water	LC50	[TOXSTUDIES0798]
257	1969	MCS 900	Rat - Inhalation	Acute (single exposure)	[TOXSTUDIES0789]
258	1969	MCS 9001	Rat - Inhalation	Acute (single exposure)	[TOXSTUDIES0832]
259	1969	Aroclor 1242	Rat - Oral	Repeated dose	[TOXSTUDIES0766]
260	1969	Aroclor 1254	Rat - Oral	Tissue burden	[TOXSTUDIES0766]
261	1969	Aroclor 1260	Rat - Oral	Tissue burden	[TOXSTUDIES0766]
262	1969	Aroclor 5460	Rat - Oral	Tissue burden	[TOXSTUDIES0766]
263	1969	Halowax 1014	Rat - Oral	Tissue burden	[TOXSTUDIES0766]
264	1969	Halowax 1099	Rat - Oral	Tissue burden	[TOXSTUDIES0766]
265	1969	MCS 528	Rat - Oral	LD50	[TOXSTUDIES0754]
266	1969	MCS 900	Rat - Oral	LD50	[TOXSTUDIES0789]
267	1969	MCS 9001	Rat - Oral	LD50	[TOXSTUDIES0832]
268	1969	Pydraul 312	Rat - Oral	LD50	[TOXSTUDIES0760]
269	1969	Toxaphene	Rat - Oral	Tissue burden	[TOXSTUDIES0766]
270	1970	Aroclor 1242	Chicken - Oral	Reproductive toxicity	[TOXSTUDIES0864]
271	1970	Aroclor 1254	Chicken - Oral	Reproductive toxicity	[TOXSTUDIES0864]
272	1970	Aroclor 1260	Chicken - Oral	Reproductive toxicity	[TOXSTUDIES0864]
273	1970	Aroclor 4273 - MCS 1004	Rabbit - Dermal	MLD	[TOXSTUDIES0965]
274	1970	Aroclor 4273 - MCS 1004	Rabbit - Dermal	Irritation	[TOXSTUDIES0965]
275	1970	Aroclor 6037 - MCS 1057-1	Rabbit - Dermal	MLD	[TOXSTUDIES0957]
276	1970	Aroclor 6037 - MCS 1057-1	Rabbit - Dermal	Irritation	[TOXSTUDIES0957]
277	1970	Aroclor 6062	Rabbit - Dermal	MLD	[TOXSTUDIES0950]
278	1970	Aroclor 6062	Rabbit - Dermal	Irritation	[TOXSTUDIES0950]

Item	Year	Study design	Species - Test article	Exposure route	Bates Number
279	1970	Aroclor 6070	Rabbit - Dermal	MLD	[TOXSTUDIES0982]
280	1970	Aroclor 6070	Rabbit - Dermal	Irritation	[TOXSTUDIES0982]
281	1970	Aroclor 6090	Rabbit - Dermal	MLD	[TOXSTUDIES0990]
282	1970	Aroclor 6090	Rabbit - Dermal	Irritation	[TOXSTUDIES0990]
283	1970	MCS 1009	Rabbit - Dermal	MLD	[TOXSTUDIES0848]
284	1970	MCS 1009	Rabbit - Dermal	Irritation	[TOXSTUDIES0848]
285	1970	MCS 999	Rabbit - Dermal	MLD	[TOXSTUDIES0856]
286	1970	MCS 999	Rabbit - Dermal	Irritation	[TOXSTUDIES0856]
287	1970	Pydraul 281	Rabbit - Dermal	MLD	[TOXSTUDIES0840]
288	1970	Pydraul 281	Rabbit - Dermal	Irritation	[TOXSTUDIES0840]
289	1970	Aroclor 4273 - MCS 1004	Rabbit - Ocular	Irritation	[TOXSTUDIES0965]
290	1970	Aroclor 6037 - MCS 1057-1	Rabbit - Ocular	Irritation	[TOXSTUDIES0957]
291	1970	Aroclor 6062	Rabbit - Ocular	Irritation	[TOXSTUDIES0950]
292	1970	Aroclor 6070	Rabbit - Ocular	Irritation	[TOXSTUDIES0982]
293	1970	Aroclor 6090	Rabbit - Ocular	Irritation	[TOXSTUDIES0990]
294	1970	MCS 1009	Rabbit - Ocular	Irritation	[TOXSTUDIES0848]
295	1970	MCS 999	Rabbit - Ocular	Irritation	[TOXSTUDIES0856]
296	1970	Pydraul 281	Rabbit - Ocular	Irritation	[TOXSTUDIES0840]
297	1970	Aroclor 4273 - MCS 1004	Rat - Inhalation	Acute (single exposure)	[TOXSTUDIES0965]
298	1970	Aroclor 6037 - MCS 1057-1	Rat - Inhalation	Acute (single exposure)	[TOXSTUDIES0957]
299	1970	Aroclor 6070	Rat - Inhalation	Acute (single exposure)	[TOXSTUDIES0982]
300	1970	MCS 1009	Rat - Inhalation	Acute (single exposure)	[TOXSTUDIES0848]
301	1970	MCS 999	Rat - Inhalation	Acute (single exposure)	[TOXSTUDIES0856]
302	1970	Pydraul 281	Rat - Inhalation	Acute (single exposure)	[TOXSTUDIES0840]
303	1970	Aroclor 4273 - MCS 1004	Rat - Oral	LD50	[TOXSTUDIES0965]
304	1970	Aroclor 6037 - MCS 1057-1	Rat - Oral	LD50	[TOXSTUDIES0957]
305	1970	Aroclor 6062	Rat - Oral	LD50	[TOXSTUDIES0950]
306	1970	Aroclor 6070	Rat - Oral	LD50	[TOXSTUDIES0982]
307	1970	Aroclor 6090	Rat - Oral	Acute (single exposure)	[TOXSTUDIES0990]
308	1970	MCS 1009	Rat - Oral	LD50	[TOXSTUDIES0848]
309	1970	MCS 999	Rat - Oral	LD50	[TOXSTUDIES0856]
310	1970	Pydraul 281	Rat - Oral	LD50	[TOXSTUDIES0840]
311	1971	Aroclor 1016	Bluegill fish - Water	TL50	[TOXSTUDIES1790]
312	1971	Aroclor 1242	Bluegill fish - Water	TL50	[TOXSTUDIES1790]
313	1971	Aroclor 1016	Catfish - Water	TL50	[TOXSTUDIES1790]
314	1971	Aroclor 1242	Catfish - Water	TL50	[TOXSTUDIES1790]

Item	Year	Study design	Species - Test article	Exposure route	Bates Number
315	1971	Aroclor 1242	Chicken - Oral	Reproductive toxicity	[TOXSTUDIES1049]
316	1971	Aroclor 1221	Dog - Oral	Repeated dose	[TOXSTUDIES1799]
317	1971	Aroclor 1242	Dog - Oral	Chronic toxicity	[TOXSTUDIES1276]
318	1971	Aroclor 1254	Dog - Oral	Chronic toxicity	[TOXSTUDIES1198]
319	1971	Aroclor 1260	Dog - Oral	Chronic toxicity	[TOXSTUDIES1351]
320	1971	Aroclor 1016 (MCS 1016)	Rabbit - Dermal	Irritation	[TOXSTUDIES1008]
321	1971	Aroclor 1016 (MCS 1016)	Rabbit - Dermal	LD50	[TOXSTUDIES1008]
322	1971	Aroclor 1221	Rabbit - Dermal	LD50	[TOXSTUDIES1008]
323	1971	Aroclor 1221	Rabbit - Dermal	Irritation	[TOXSTUDIES1008]
324	1971	Aroclor 1272	Rabbit - Dermal	MLD	[TOXSTUDIES1085]
325	1971	Aroclor 1272	Rabbit - Dermal	Irritation	[TOXSTUDIES1085]
326	1971	Aroclor 5442	Rabbit - Dermal	LD50	[TOXSTUDIES1008]
327	1971	Aroclor 5442	Rabbit - Dermal	Irritation	[TOXSTUDIES1008]
328	1971	Aroclor 1016 (MCS 1016)	Rabbit - Ocular	Irritation	[TOXSTUDIES1008]
329	1971	Aroclor 1221	Rabbit - Ocular	Irritation	[TOXSTUDIES1008]
330	1971	Aroclor 1272	Rabbit - Ocular	Irritation	[TOXSTUDIES1085]
331	1971	Aroclor 5442	Rabbit - Ocular	Irritation	[TOXSTUDIES1008]
332	1971	Aroclor 1016 (MCS 1016)	Rat - Inhalation	Acute (single exposure)	[TOXSTUDIES1002]
333	1971	Aroclor 1016 (MCS 1016)	Rat - Oral	LD50	[TOXSTUDIES1008]
334	1971	Aroclor 1221	Rat - Oral	LD50	[TOXSTUDIES1008]
335	1971	Aroclor 1242	Rat - Oral	Teratology	[TOXSTUDIES1111]
336	1971	Aroclor 1242	Rat - Oral	Reproductive toxicity	[TOXSTUDIES1147]
337	1971	Aroclor 1242	Rat - Oral	Chronic toxicity	[TOXSTUDIES1702]
338	1971	Aroclor 1254	Rat - Oral	Teratology	[TOXSTUDIES1091]
339	1971	Aroclor 1254	Rat - Oral	Reproductive toxicity	[TOXSTUDIES1426]
340	1971	Aroclor 1254	Rat - Oral	Chronic toxicity	[TOXSTUDIES1612]
341	1971	Aroclor 1260	Rat - Oral	Teratology	[TOXSTUDIES1129]
342	1971	Aroclor 1260	Rat - Oral	Reproductive toxicity	[TOXSTUDIES1477]
343	1971	Aroclor 1260	Rat - Oral	Chronic toxicity	[TOXSTUDIES1525]
344	1971	Aroclor 1272	Rat - Oral	MLD	[TOXSTUDIES1085]
345	1971	Aroclor 5442	Rat - Oral	LD50	[TOXSTUDIES1008]
346	1972	Aroclor 1016 (MCS 1016)	Bluegill fish - Water	TL50	[TOXSTUDIES1873]
347	1972	Aroclor 1221	Bluegill fish - Water	TL50	[TOXSTUDIES1873]
348	1972	Aroclor 1242	Bluegill fish - Water	TL50	[TOXSTUDIES2137]
349	1972	Aroclor 1254	Bluegill fish - Water	TL50	[TOXSTUDIES2137]
350	1972	Aroclor 1260	Bluegill fish - Water	TL50	[TOXSTUDIES2137]
351	1972	Aroclor 5432	Bluegill fish - Water	TL50	[TOXSTUDIES1873]
352	1972	Aroclor 5442	Bluegill fish - Water	TL50	[TOXSTUDIES1873]
353	1972	Aroclor 5460	Bluegill fish - Water	TL50	[TOXSTUDIES1873]

Item	Year	Study design	Species - Test article	Exposure route	Bates Number
354	1972	Aroclor 1016 (MCS 1016)	Catfish - Water	TL50	[TOXSTUDIES1873]
355	1972	Aroclor 1221	Catfish - Water	TL50	[TOXSTUDIES1873]
356	1972	Aroclor 1242	Catfish - Water	TL50	[TOXSTUDIES2137]
357	1972	Aroclor 1254	Catfish - Water	TL50	[TOXSTUDIES2137]
358	1972	Aroclor 1260	Catfish - Water	TL50	[TOXSTUDIES2137]
359	1972	Aroclor 5432	Catfish - Water	TL50	[TOXSTUDIES1873]
360	1972	Aroclor 5442	Catfish - Water	TL50	[TOXSTUDIES1873]
361	1972	Aroclor 5460	Catfish - Water	TL50	[TOXSTUDIES1873]
362	1972	Aroclor 1016 (MCS 1016)	Chicken - Oral	Reproductive toxicity	[TOXSTUDIES2081]
363	1972	Aroclor 1221	Chicken - Oral	Reproductive toxicity	[TOXSTUDIES2109]
364	1972	Aroclor 1016 (MCS 1016)	Dog - Oral	Repeated dose	[TOXSTUDIES1962]
365	1972	Aroclor 1242	Mouse - Intraperitoneal	Acute (single exposure)	[TOXSTUDIES1944]
366	1972	Aroclor 1242	Mouse - Intraperitoneal	Dominant lethal mutagenicity	[TOXSTUDIES1944]
367	1972	Aroclor 1254	Mouse - Intraperitoneal	Acute (single exposure)	[TOXSTUDIES1928]
368	1972	Aroclor 1254	Mouse - Intraperitoneal	Dominant lethal mutagenicity	[TOXSTUDIES1928]
369	1972	Aroclor 1260	Mouse - Intraperitoneal	Acute (single exposure)	[TOXSTUDIES1911]
370	1972	Aroclor 1260	Mouse - Intraperitoneal	Dominant lethal mutagenicity	[TOXSTUDIES1911]
371	1972	MCS 1230	Rabbit - Dermal	MLD	[TOXSTUDIES1902]
372	1972	MCS 1230	Rabbit - Dermal	Irritation	[TOXSTUDIES1902]
373	1972	MCS 1230	Rabbit - Ocular	Irritation	[TOXSTUDIES1902]
374	1972	MCS 1230	Rat - Inhalation	Acute (single exposure)	[TOXSTUDIES1902]
375	1972	MCS 1230	Rat - Inhalation	Acute (single exposure)	[TOXSTUDIES1902]
376	1972	Aroclor 1016 (MCS 1016)	Rat - Oral	Repeated dose	[TOXSTUDIES2017]
377	1972	Aroclor 1221	Rat - Oral	Repeated dose	[TOXSTUDIES2049]
378	1972	MCS 1230	Rat - Oral	MLD	[TOXSTUDIES1902]
379	1974	Aroclor 6040	Rabbit - Dermal	MLD	[TOXSTUDIES0974]
380	1974	Aroclor 6040	Rabbit - Dermal	Irritation	[TOXSTUDIES0974]
381	1974	Aroclor 6040	Rabbit - Ocular	Irritation	[TOXSTUDIES0974]
382	1974	Aroclor 6040	Rat - Inhalation	Acute (single exposure)	[TOXSTUDIES0974]
383	1974	Aroclor 6040	Rat - Oral	LD50	[TOXSTUDIES0974]
384	0	Aroclor 1242	Bluegill fish - Water	TL50	[TOXSTUDIES1871]
385	0	Aroclor 1242	Rainbow trout - Water	TL50	[TOXSTUDIES1871]
386	0	Aroclor 1260	Rainbow trout - Water	TL50	[TOXSTUDIES1869]

Item	Year	Study design	Species - Test article	Exposure route	Bates Number
387	0	Aroclor 1242, Aroclor 1254, Aroclor 1260	Rat, Dog, chicken - Oral	Review of Acute, Repeated dose, Chronic, and Reproductive toxicity	[TOXSTUDIE50996]
388			-		
389	1963	Therminol 77	Rat - Inhalation	Acute (single dose)	[PCB-ARCH0089812]
390	1963	Therminol 77 (held at elevated temperature)	Rat - Inhalation	Acute (single dose)	[PCB-ARCH0089812]
391	1963	Therminol 77	Rabbit - Dermal	Irritation	[PCB-ARCH0089812]
392	1963	Therminol 77	Rabbit - Ocular	Irritation	[PCB-ARCH0089812]
393	1963	Therminol 77	Rat - Oral	LD50	[PCB-ARCH0089812]
394	1963	Therminol 77	Rabbit - Dermal	MLD	[PCB-ARCH0089812]
395	1970	Pydraul 625-A (MCS 975)	Rat - Inhalation	Acute (single exposure)	[PCB-ARCH0396162]
396	1970	Pydraul AC-A (MCS 976)	Rat - Inhalation	Acute (single exposure)	[PCB-ARCH0623179]
397	1970	Pydraul AC-A (Winter Grade 28)	Rat - Inhalation	Acute (single exposure)	[PCB-ARCH0396146]
398	1970	Pydraul 625-A (MCS 975)	Rabbit - Dermal	Irritation	[PCB-ARCH0396162]
399	1970	Pydraul AC-A (MCS 976)	Rabbit - Dermal	Irritation	[PCB-ARCH0623179]
400	1970	Pydraul AC-A (Winter Grade 28)	Rabbit - Dermal	Irritation	[PCB-ARCH0396146]
401	1970	Pydraul 625-A (MCS 975)	Rabbit - Ocular	Irritation	[PCB-ARCH0396162]
402	1970	Pydraul AC-A (MCS 976)	Rabbit - Ocular	Irritation	[PCB-ARCH0623179]
403	1970	Pydraul AC-A (Winter Grade 28)	Rabbit - Ocular	Irritation	[PCB-ARCH0396146]
404	1970	Pydraul 625-A (MCS 975)	Rat - Oral	LD50	[PCB-ARCH0396162]
405	1970	Pydraul AC-A (MCS 976)	Rat - Oral	LD50	[PCB-ARCH0623179]
406	1970	Pydraul AC-A (Winter Grade 28)	Rat - Oral	LD50	[PCB-ARCH0396146]
407	1970	Pydraul 625-A (MCS 975)	Rabbit - Dermal	MLD	[PCB-ARCH0396162]
408	1970	Pydraul AC-A (MCS 976)	Rabbit - Dermal	MLD	[PCB-ARCH0623179]
409	1970	Pydraul AC-A (Winter Grade 28)	Rabbit - Dermal	MLD	[PCB-ARCH0396146]
410	1971	Aroclor 1272	Rabbit - Dermal	Irritation	[PCB-ARCH0396343]
411	1971	Aroclor 1272	Rabbit - Ocular	Irritation	[PCB-ARCH0396343]
412	1971	Aroclor 1272	Rabbit - Dermal	MLD	[PCB-ARCH0396343]
413	1971	Aroclor 1272	Rat - Oral	MLD	[PCB-ARCH0396343]
414	1972	Therminol 77	Rabbit - Dermal	Irritation	[PCB-ARCH0396372]
415	1972	Therminol 77	Rabbit - Eye	Irritation	[PCB-ARCH0396372]
416	1972	Therminol 77	Rabbit - Dermal	MLD	[PCB-ARCH0396372]

Item	Year	Study design	Species - Test article	Exposure route	Bates Number
417	1972	Therminol 77	Rat - Oral	MLD	[PCB-ARCH0396372]
418	1973	MCS 1489	Rat - Inhalation	Acute (single exposure)	[PCB-ARCH0445483]
419	1973	MCS 1489	Rabbit - Dermal	Irritation	[PCB-ARCH0445483]
420	1973	MCS 1489	Rabbit - Ocular	Irritation	[PCB-ARCH0445483]
421	1973	MCS 1489	Rat - Oral	LD50	[PCB-ARCH0445483]
422	1973	MCS 1489	Rabbit - Dermal	MLD	[PCB-ARCH0445483]
423	1974	MCS 1043	Rat - Inhalation	Acute (single exposure)	[PCB-ARCH0445359]
424	1974	MCS 1043	Rabbit - Dermal	Irritation	[PCB-ARCH0445359]
425	1974	MCS 1717	Rabbit - Dermal	Irritation	[PCB-ARCH0445361]
426	1974	MCS 1043	Rabbit - Ocular	Irritation	[PCB-ARCH0445359]
427	1974	MCS 1717	Rabbit - Ocular	Irritation	[PCB-ARCH0445361]
428	1974	MCS 1043	Rabbit - Dermal	LD50	[PCB-ARCH0445359]
429	1974	MCS 1717	Rabbit - Dermal	LD50	[PCB-ARCH0445361]
430	1974	MCS 1043	Rat - Oral	LD50	[PCB-ARCH0445359]
431	1974	MCS 1717	Rat - Oral	LD50	[PCB-ARCH0445361]
432	1974	Aroclor 1016	Rat - Oral	Repeated dose	[PCB-ARCH0252565]
433	1974	Aroclor 1242	Rat - Oral	Repeated dose	[PCB-ARCH0252565]
434	1974	MCS 1043	Rat - Oral	Repeated dose	[PCB-ARCH0252565]
435	1974	MCS 1489 (Aroclor 1016 fraction)	Rat - Oral	Repeated dose	[PCB-ARCH0252565]
436	1974	Monochlorobiphenyl	Rat - Oral	Repeated dose	[PCB-ARCH0252565]
437	1974	MCS 1238	Bluegills - Aquatic	TL50 (4-day)	[PCB-ARCH0445346]
438	1974	MCS 1475	Bluegills - Aquatic	TL50 (4-day)	[PCB-ARCH0445348]
439	1974	MCS 1238	Rainbow trout - Aquatic	TL50 (4-day)	[PCB-ARCH0445346]
440	1974	MCS 1475	Rainbow trout - Aquatic	TL50 (4-day)	[PCB-ARCH0445348]
441	1975	Aroclor 1016	Rabbit - Dermal	Irritation	[PCB-ARCH0070083]
442	1975	Aroclor 1016	Rabbit - Ocular	Irritation	[PCB-ARCH0070083]
443	1975	Aroclor 1016	Rabbit - Dermal	LD50	[PCB-ARCH0070083]
444	1975	Aroclor 1016	Rat - Oral	LD50	[PCB-ARCH0070083]
445	1981	Aroclor 1254	Monkey - Oral	Repeated dose	[PCB-ARCH0638056]
446	1981	PCBs	Mouse, rat, human - Review	Review	[PCB-ARCH0617804]
447	1986	Aroclor 1254	Cow - Oral	Repeated dose	[PCB-ARCH0662330]
448	0	PCBs	Human - Occupational	Epidemiology-prospective mortality	[PCB-ARCH0715727]
449	0	PCBs	Human - Occupational	Epidemiology-retrospective mortality	[PCB-ARCH0675078]
450	0	PCBs	Human - Occupational	Epidemiology review-various morbidity	[PCB-ARCH0683351]
451	0	Aroclor 1260	Rat - Oral	Repeated dose	[PCB-ARCH0029231]
452	0	Aroclor 1260	Rainbow trout - Aquatic	TL50 (4-day)	[PCB-ARCH0636488]

2. Attachment 1B. List of PCB-related analytical studies conducted under contract from Monsanto

Item	Date	Title*
453	1/1/1968	FINAL REPORT ON ANALYTICAL CHEMISTRY AND SPECTROSCOPY INVESTIGATIONS - 1967 PART I - ANALYTICAL METHODS DEVELOPED REPORT NO 3513 JOB NO 2-02-760.01-6830-I (see 67-5; 67-18)
454	1/1/1968	FINAL REPORT ON ANALYTICAL CHEMISTRY AND SPECTROSCOPY INVESTIGATIONS - 1967 PART II - SPECIAL STUDIES REPORT NO 3514 JOB NO 2-20-760.01-6830-II 2-02-760.01-6520 2-20-760.01-6313 2-02-760.01-6308 (see 67-16; 67-18; 67-23; 67-33)
455	5/1/1968	REPORT ON THE MAY, 1968, SURVEY OF CHOCCOLOCCO CREEK AND TRIBUTARIES: BIOLOGY OF FISHES AND WATER QUALITY
456	1/1/1969	PROPOSAL FOR AN INDUSTRY STUDY OF THE LAKE MICHIGAN BASIN
457	1/1/1969	REPORT ON THE JANUARY, 1969, SURVEY OF CHOCCOLOCCO CREEK AND TRIBUTARIES: BIOLOGY OF FISHES AND WATER QUALITY
458	1/1/1969	FINAL REPORT ON SPECTROSCOPY METHODS DEVELOPED - 1968 REPORT NO 3549 JOB NO 2-02-760.01-8903209 (PARTIAL REPORT ONLY CONTAINS INDEX, INTRODUCTION, SECTION TITLES AND SUMMARY) (see 68-3)
459	9/1/1969	FINAL REPORT ON ANALYTICAL CHEMISTRY INVESTIGATIONS - 1968 METHOD DEVELOPMENT - GENERAL UTILITY (PARTIAL REPORT CONTAINS ONLY INDEX, SECTION TITLES, INTRODUCTION AND SUMMARY) Report No. 3613 (see 68-13)
460	9/15/1969	SUMMARY OF RESULTS AS OF SEPTEMBER 8, 1969 (WATER SAMPLES FROM LAKE MICHIGAN)
461	9/15/1969	FINAL REPORT ON GAS CHROMATOGRAPHY METHOD DEVELOPMENT - 1968 (PARTIAL REPORT ONLY CONTAINS INDEX, SECTION TITLES, INTRODUCTION, AND SUMMARY) (see 68-10; 68-18; 68-19) Report 3598
462	10/1/1969	REPORT ON THE OCTOBER 1969 SURVEY OF CHOCCOLOCCO CREEK AND TRIBUTARIES: FISHES AND WATER QUALITY (STUDY TO ENHANCE GENERAL KNOWLEDGE OF FISH FAUNA, DETERMINE WATER QUALITY, STUDY STREAM GEOGRAPHY, AND COLLECT ANIMAL SAMPLES FOR MONSANTO RESIDUE ANALYSIS)
463	1/1/1970	PROGRESS REPORT I LAKE MICHIGAN PESTICIDE INVESTIGATIONS
464	1/1/1970	FINAL REPORT ON SPECIAL SPECTROSCOPY STUDIES 1969 (PARTIAL REPORT CONTAINS ONLY INTRODUCTION, SECTION TITLES, INDEX AND SUMMARY) (see 69-5) Report 3630
465	2/1/1970	IDENTIFICATION OF POLYCHLORINATED BIPHENYLS BY GAS CHROMATOGRAPHY/ MASS SPECTROMETRY SPECTROSCOPY METHOD 70-1 Report 3684
466	2/16/1970	GAS CHROMATOGRAPHIC DETERMINATION OF 4-CHLOROBIPHENYL IN AROCLOR 1221 PHYSICAL CHEMISTRY METHOD NO. 70-1 (INCLUDES ADDENDUM) Report 3714
467	3/1/1970	STUDY OF AROCLOR CHEMICAL CLEAN-UP PROCEDURES FOR USE WITH ELECTRON CAPTURE GAS CHROMATOGRAPHY METHODS ANALYTICAL CHEMISTRY SPECIAL STUDY 70-4 Report 3688
468	3/1/1970	SOLUBILITY OF AROCLOR 1242, 1248, AND 1254 IN WATER ANALYTICAL CHEMISTRY SPECIAL STUDY 70-5 Report 3688
469	3/1/1970	STUDY OF THE OPEN AIR COMBUSTION OF PAPER CONTAINING AROCLOR 1242 ANALYTICAL CHEMISTRY SPECIAL STUDY 70-6 Report 3688
470	3/1/1970	THE PARTITION OF POLYCHLORINATED BIPHENYLS BETWEEN HEXANE AND VARIOUS ORGANIC POLAR SOLVENTS ANALYTICAL CHEMISTRY SPECIAL STUDY 70-7 Report 3688

Item	Date	Title*
471	3/1/1970	EXTRACTION OF AROCLOR 1254 FROM PAINTED SURFACES BY WATER ANALYTICAL CHEMISTRY SPECIAL STUDY 70-8 Report 3688
472	4/1/1970	STUDY OF THE COMPLETENESS OF THERMAL OXIDATION OF POLYCHLORINATED BIPHENYLS AT JOHN ZINK POLLUTION RESEARCH ANALYTICAL CHEMISTRY SPECIAL STUDY 70-12 Report 3688
473	4/1/1970	RETENTION TIMES AND ELECTRON CAPTURE RESPONSE FACTORS OF SOME POLYCHLORINATED BIPHENYL ISOMERS RELATIVE TO DDE ANALYTICAL CHEMISTRY SPECIAL STUDY 70-13 Report 3688
474	4/1/1970	GAS CHROMATOGRAPHIC DETERMINATION OF LOW BOILERS IN RECLAIMED THERMINOL FR-1 AND FR-2 PHYSICAL CHEMISTRY METHOD NO. 70-12 Report 3714
475	4/1/1970	DETERMINATION OF PCB RESIDUES IN FISH FROM LAKE MICHIGAN ANALYTICAL CHEMISTRY SPECIAL STUDY 70-11 Report 3688
476	7/1/1970	MONOISOPROPYLBIPHENYL PHYSICAL PROPERTY DETERMINATIONS (PHYSICAL CHEMISTRY SPECIAL STUDY 70-28) Addendum 9/1970 Report 3713
477	7/17/1970	FINAL REPORT ON GAS CHROMATOGRAPHY METHOD DEVELOPMENT - 1969 (PARTIAL REPORT CONTAINS ONLY THE INDEX, SECTION TITLES, INTRODUCTION AND SUMMARY) (see 69-13) Report 3658
478	9/1/1970	DETERMINATION OF POLYCHLORINATED BIPHENYL RESIDUES IN RATS FROM 30 DAY AROCLOR FEEDING STUDIES ANALYTICAL CHEMISTRY SPECIAL STUDY 70-17 Report 3688
479	11/1/1970	ANALYSIS OF WATER AND SEDIMENT FOR POLYCHLORINATED BIPHENYLS ANALYTICAL CHEMISTRY METHOD 69-13 Report 3677
480	11/1/1970	ANALYSIS OF BIODEGRADATION DATA STATISTICS SPECIAL STUDY 70-22 Report 3682
481	11/1/1970	CHLORINE DISTRIBUTION OF AROCLOR PRODUCTS BY PACKED COLUMN, ELECTRON CAPTURE, GAS CHROMATOGRAPHY/MASS SPECTROMETRY ANALYTICAL CHEMISTRY SPECIAL STUDY 70-18 Report 3688
482	11/1/1970	A TENTATIVE PROCEDURE FOR THE DETERMINATION OF AIRBORNE-POLYCHLORINATED BIPHENYLS ANALYTICAL CHEMISTRY METHOD 70-6 Report 3687
483	11/1/1970	ANALYSIS OF BIOLOGICAL MATERIALS FOR POLYCHLORINATED BIPHENYLS ANALYTICAL CHEMISTRY METHOD 70-1 Report 3687
484	12/1/1970	AROCLOR DEFENSE - 1970 PCB SUPPORT ANALYSIS ANALYTICAL CHEMISTRY SPECIAL STUDY 70-21 Report 3688
485	12/1/1970	PARTICLE SIZE DETERMINATION ON ANNISTON LIMESTONE PIT WASTE EFFLUENT SOLIDS PHYSICAL CHEMISTRY SPECIAL STUDY 70-44 (INCLUDES POLAROID PHOTOMICROGRAPHS OF THE PIT EFFLUENT SOLIDS) Report 3713
486	1/1/1971	FINAL REPORT ON ANALYTICAL CHEMISTRY INVESTIGATIONS - 1970 SPECIAL STUDIES (PARTIAL REPORT CONTAINS ONLY INDEX, SECTION TITLES, INTRODUCTION AND SUMMARY) (see 70-4; 70-5; 70-6; 70-8; 70-11; 70-13; 70-17; 70-18; 70-21) Report 3688
487	1/1/1971	FINAL REPORT ON ANALYTICAL CHEMISTRY INVESTIGATIONS - 1970 METHOD DEVELOPMENT - GENERAL UTILITY (PARTIAL REPORT CONTAINS ONLY INDEX, SECTION TITLES, INTRODUCTION AND SUMMARY) (see 70-1; 70-6) Report 3687
488	1/1/1971	FINAL REPORT ON SPECTROSCOPY METHODS DEVELOPED - 1970 (PARTIAL REPORT - ONLY CONTAINS METHOD INDEX); Report No. 3684 (see 70-1: 70-12; 70-16; 70-17; 70-18; 70-19; 70-23; 70-25)
489	1/22/1971	COMPOSITION OF COMPETITIVE POLYCHLORINATED BIPHENYL PRODUCTS MEMO, R.E. KELLER TO A.F. REGAN, 11-5-70 (COVER FOR RESULTS OF ISOMER ANALYSES) Report 3784 71-3)

Item	Date	Title*
490	2/1/1971	MCS 1016 - AN ENVIRONMENTALLY COMPATIBLE AROCLOR ANALYTICAL CHEMISTRY SPECIAL STUDY 71-2 Report 3819
491	3/1/1971	DETERMINATION OF POLYCHLORINATED BIPHENYL RESIDUES IN WHITE LEGHORN CHICKENS FROM A TOXICITY, REPRODUCTION AND RESIDUE STUDY WITH AROCLOR 1242, AROCLOR 1254, AND AROCLOR 1260 ANALYTICAL CHEMISTRY SPECIAL STUDY 71-3 Report 3819
492	3/1/1971	ANALYSIS OF ANNISTON PLANT WASTE EFFLUENT FOR TRACE ORGANIC COMPONENT SPECTROSCOPY SPECIAL STUDY 71-14 Report 3785
493	3/1/1971	STUDY OF OPERATING VARIABLES IN THE ACTIVATED SLUDGE BIODEGRADABILITY TEST STATISTICS SPECIAL STUDY 71-2 Report 3760
494	4/1/1971	COMPOSITION OF CHLORINATED BIPHENYL PRODUCTS IN THE RANGE FROM 8 TO 42% CHLORINATION LEVEL 71-11 Job no. 1711022 Report 3784
495	5/1/1971	GAS CHROMATOGRAPHIC DETERMINATION OF PENTA- AND HEXA - CHLOROBIPHENYLS IN AROCLOR 1242 AND MCS 1016 PHYSICAL CHEMISTRY METHOD NO. 71-11 Report 3783
496	5/13/1971	IMPROVED METHOD FOR THE GAS CHROMATOGRAPHIC DETERMINATION OF CHLORINATED BIPHENYL ISOMERS IN AROCLOR PRODUCTS PHYSICAL CHEMISTRY METHOD NO. 70-20 Report 3714
497	6/1/1971	DETERMINATION OF AROCLORS AND RELATED PRODUCTS IN MIXED LIQUOR SAMPLES FROM SEMICONTINUOUS ACTIVATED SLUDGE UNITS BY ULTRAVIOLET ABSORPTION (71-17) Report 3820
498	8/1/1971	BIODEGRADATION STUDIES COMPARISON OF MCS 1016 WITH AROCLOR 1142 RESIDUE STATISTICS SPECIAL STUDY 71-12 Report 3760
499	9/16/1971	MONSANTO COMPANY, FOURTH QUARTERLY TRIP, FIRST YEAR OF SURVEY (FISHES FOR RESIDUE ANALYSIS; TURNED OVER TO MONSANTO COMPANY - SUMMARY OF TOTAL COLLECTED)
500	10/1/1971	ANALYSIS OF ENVIRONMENTAL MATERIALS FOR POLYCHLORINATED BIPHENYLS (METHOD 71-35) Report 3820
501	10/1/1971	SUGGESTED PROCEDURE FOR BIODEGRADABILITY SCREENING STATISTICS SPECIAL STUDY 71-17 Report 3760
502	10/1/1971	DETERMINATION OF POLYCHLORINATED BIPHENYL RESIDUES IN ALBINO RATS FROM A TWO-YEAR CHRONIC ORAL TOXICITY STUDY ANALYTICAL CHEMISTRY SPECIAL STUDY 71-7 Report 3819
503	10/1/1971	GAS CHROMATOGRAPHIC DETERMINATION OF PENTA- AND HEXA- CHLOROBIPHENYLS IN AROCLOR 1016 AND AROCLOR 1242 (METHOD NO. 71-11A) Report 3783
504	10/26/1971	GAS CHROMATOGRAPHIC ANALYSIS DETERMINATION OF 4,4 -DICHLOROBIPHENYL IN AROCLOR PRODUCTS PHYSICAL CHEMISTRY METHOD NO. 71-18 Report 3783
505	12/1/1971	ANALYSIS OF RECOVERED PYDRAUL 312 FROM CENTRAL FOUNDRY SPECTROSCOPY SPECIAL STUDY 71-31 Report 3785
506	12/1/1971	PCB RESIDUES IN BEAGLE DOGS 71-25 Job no. 8505016 Report 3760
507	12/1/1971	BIODEGRADATION TESTING OF POLYCHLORINATED BIPHENYLS ANALYTICAL CHEMISTRY SPECIAL STUDY 71-6 Report 3819
508	12/1/1971	PERCHLORINATION OF POLYCHLORINATED BIPHENYLS AND TERPHENYLS PRIOR TO ELECTRON CAPTURE GAS CHROMATOGRAPHY 71-1 Job no. 1348006 8501006 Report 3804

Item	Date	Title*
509	12/9/1971	MONSANTO COMPANY, FIRST QUARTERLY TRIP, SECOND YEAR OF SURVEY (FISHES FOR RESIDUE ANALYSIS; TURNED OVER TO MONSANTO COMPANY, DECEMBER 9, 1971.)
510	1/1/1972	POLYCHLORINATED BIPHENYL BIODEGRADATION STUDIES
511	1/1/1972	FINAL PROGRESS REPORT TO MONSANTO COMPANY, RESIDUE DATA, NOVEMBER, 1970 TO NOVEMBER, 1972 (FISH)
512	1/1/1972	FINAL REPORT ON SPECIAL SPECTROSCOPY STUDIES 1971 (PARTIAL REPORT CONTAINS ONLY INDEX, SECTION TITLES, INTRODUCTION AND SUMMARY) (see 71-14; 71-31) Report 3785
513	3/1/1972	FINAL REPORT ON STATISTICAL APPLICATIONS – 1971 SPECIAL STUDIES (ALBINO RATS, LEGHORN CHICKENS AND BEAGLE DOGS) (PARTIAL REPORT CONTAINS ONLY INDEX, SECTION TITLES, INTRODUCTION AND SUMMARY) (see 71-2; 71-12; 71-17; 71-25) Report 3760
514	3/1/1972	NATIONAL BROILER COUNCIL INTERLABORATORY PCB METHODOLOGY CHECK 72-6 Job no. 8505016 Report 3818
515	3/1/1972	ROUND ROBIN STUDY - PCB IN WATER 72-5 Job no. 8505016 Report 3818
516	3/8/1972	(PROPOSAL FOR ENVIRONMENTAL PERSISTENCE STUDIES WITH PCBs)
517	4/1/1972	POLYCHLORINATED BIPHENYL BIODEGRADATION (SEMI-CONTINUOUS ACTIVATED SLUDGE INCLUDES CHARTS AND GRAPHS) BLUE GILLS CATFISH, LEGHORN CHICKENS, ALBINO RATS, BEAGLE DOGS)
518	5/30/1972	MONSANTO COMPANY, SECOND QUARTERLY TRIP, SECOND YEAR OF SURVEY MAY 30-JUNE 1, 1972 PART II - FISHES FOR ICHTHYOLOGICAL SURVEY (LIST OF SPECIES AND LOCAL)
519	6/9/1972	(RESIDUE DATA ON VARIOUS FISH SPECIES AROUND THE ANNISTON PLANT)
520	9/14/1972	FISHES FOR ICHTHYOLOGICAL SURVEY (FOURTH QUARTERLY TRIP, SECOND YEAR OF SURVEY)
521	10/1/1972	MONSANTO COMPANY, FOURTH QUARTERLY TRIP, SECOND YEAR OF SURVEY SEPTEMBER 12-14, 1972 PART I - FISHES FOR RESIDUE ANALYSIS; TURNED OVER TO MONSANTO COMPANY, SEPTEMBER 13-14, 1972 (LIST OF FISH WITH SCIENTIFIC AND COMMON NAMES, AND SAMPLE SITES)
522	11/1/1972	FINAL PROGRESS REPORT TO MONSANTO COMPANY, RESIDUE DATA, NOVEMBER, 1970 TO NOVEMBER (FISH)
523	12/1/1972	DETERMINATION OF POLYCHLORINATED BIPHENYL RESIDUES IN BEAGLE DOG TISSUE FROM A TWO- YEAR CHRONIC ORAL TOXICITY STUDY ANALYTICAL CHEMISTRY SPECIAL STUDY 71-9 Report 3819
524	12/1/1972	AROCLO 1016 SOIL MIGRATION STUDIES ANALYTICAL CHEMISTRY SPECIAL STUDY 72-6 Report 3895
525	12/1/1972	DETERMINATION OF POLYCHLORINATED BIPHENYLS IN MHA ANALYTICAL CHEMISTRY METHOD NO. 71-27 Report 3820
526	1/1/1973	THE EVALUATION OF BARRIER COATINGS IN FARM SILOS WHICH WERE PCB-CONTAMINATED
527	1/1/1973	DECONTAMINATION OF SILOS CONTAMINATED WITH POLYCHLORINATED BIPHENYLS (PCB'S) –SOME IMPORTANT CONSIDERATIONS
528	1/1/1973	THE DEGRADATION OF POLYCHLORINATED BIPHENYLS BY MICRO-ORGANISMS (INCLUDES GRAPHS)
529	1/1/1973	MIGRATION OF POLYCHLORINATED BIPHENYLS IN SOIL INDUCED BY PERCOLATING WATER

Item	Date	Title*
530	1/1/1973	FINAL PROGRESS REPORT TO MONSANTO COMPANY, RESIDUE DATA, NOVEMBER, 1970 TO NOVEMBER, 1972
531	1/1/1973	BIODEGRADATION TESTING OF AROCLOR 1016 AND AROCLOR 1142 DISTILLATION RESIDUE ANALYTICAL CHEMISTRY SPECIAL STUDY 71-1 Report 3819
532	3/1/1973	ANALYTICAL CHEMISTRY INVESTIGATIONS – 1971 METHOD DEVELOPMENT - GENERAL UTILITY (POULTRY, FISH & ALBINO RATS) (PARTIAL REPORT CONTAINS ONLY THE INDEX, SECTION TITLES, INTRODUCTION AND SUMMARY) (see 71-17: 71-27: 71-35) Report 3820
533	3/1/1973	ANALYTICAL CHEMISTRY INVESTIGATIONS – 1971 SPECIAL STUDIES (ALBINO RATS, WHITE LEGHORN CHICKENS, & BEAGLE DOGS) (PARTIAL REPORT CONTAINS ONLY THE INDEX, SECTION TITLES, INTRODUCTION AND SUMMARY) Report No. 3819 (see 71-6; 71-7; 71-9) Report 3819
534	6/7/1973	“Report to IBT Research - Toxicity and Reproduction Study with Aroclor 1242 in White Leghorn Chickens” Bio-Test BT-73-70 (J1291)
535	9/10/1973	REPORT (ANALYSIS FOR PCB)
536	10/1/1973	DETERMINATION OF POLYCHLORINATED BIPHENYLS IN THERMINOL 55 BY ELECTRON CAPTURE GAS CHROMATOGRAPHY 73-8 Job no.1048006 Report 3935
537	10/1/1973	DETERMINATION OF POLYCHLORINATED BIPHENYLS IN THERMINOL 66 BY ELECTRON CAPTURE GAS CHROMATOGRAPHY 73-9 Job no. 1048006 Report 3935
538	12/1/1973	COATINGS AS BARRIERS TO PREVENT POLYCHLORINATED BIPHENYL CONTAMINATION OF SILAGE
539	1/1/1974	INVERESK STUDIES (MODEL ECOSYSTEM, INCLUDING FISH, MOLLUSCA, WEED, CRUSTACEA, MUD & ALGAE; CONSUMPTION OF A-1254 & A-1242; MOVEMENT OF PCBS IN ECOSYSTEM) with attached graph
540	1/1/1974	RUABON POND EXPERIMENTS
541	1/1/1974	BIODEGRADATION OF POLYCHLORINATED BIPHENYLS
542	1/1/1974	DETERMINATION OF MONOCHLOROBIPHENYL IN SEMICONTINUOUS ACTIVATED SLUDGE MIXED LIQUOR SAMPLES 73-24 Job no. 1020006 Report 3935
543	6/8/1974	Animal Feeding Studies for Tissue Residue Analysis Younger Y-74-46-Y-74-55 (mix PCB and non-PCB)
544	6/12/1974	SPECIAL SURVEY—COLLECTION OF FISHES FOR RESIDUE ANALYSIS
545	7/1/1974	GAS CHROMATOGRAPHIC DETERMINATION OF CHLORINATED BIPHENYL ISOMERS IN AROCLOR PRODUCTS 73-19 Job no. 1016022 Report 3908
546	8/1/1974	DETERMINATION OF THE WEIGHT PERCENT HOMOLOG COMPOSITION OF AROCLOR 1016 ANALYTICAL CHEMISTRY SPECIAL STUDY 74-4 Report 3968
547	8/1/1974	BIODEGRADABILITY OF AROCLOR 1221 ANALYTICAL CHEMISTRY SPECIAL STUDY 72-17 Report 3895
548	9/1/1974	BIODEGRADATION DIE-AWAY TESTING OF AROCLOR 1016, AROCLOR 1242 AND AROCLOR 1142 DISTILLATION RESIDUES 72-19 Job no. 1650006 Report 3895
549	9/25/1974	(TRANSMITTAL OF PCB RESIDUE DATA FROM FISH CAUGHT NEAR THE ANNISTON PLANT)
550	10/4/1974	(INFORMATION ON DEFORMITIES IN GOLDFISH AND SUNFISH EXPOSED TO PCBS NEAR ANNISTON PLANT)
551	10/25/1974	(ANALYSIS OF RESIDUE DATA ON VARIOUS SPECIES OF FISH)

Item	Date	Title*
552	11/1/1974	ANALYTICAL CHEMISTRY INVESTIGATIONS - 1972 SPECIAL STUDIES REPORT NO 3895 JOB NO 43-000-760.21-85010 (PARTIAL REPORT CONTAINS ONLY THE INDEX, SECTION TITLES, INTRODUCTION AND SUMMARY) (see 72-6; 72-17; 72-19) Report 3895
553	1/1/1975	TISSUE RESIDUES FROM SUB ACUTE ORAL FEEDING OF POLYCHLORINATED BIPHENYL DIELECTRIC FLUIDS (ALBINO RATS)
554	1/1/1975	DETERMINATION OF POLYCHLORINATED BIPHENYL RESIDUES IN ALBINO RAT AND BEAGLE DOG TISSUES FROM A 90 DAY SUBACUTE ORAL TOXICITY STUDY ANALYTICAL CHEMISTRY SPECIAL STUDY 73-26 (DISCUSSES IBT TEST NUMBERS 9888C, 9888D, 9885 AND 9887) Report 3967
555	1/1/1975	INVERESK STUDIES (CONSUMPTION OF VARIOUS PCB PRODUCTS IN MODEL ECOSYSTEMS)
556	3/14/1975	THE DEGRADATION OF POLYCHLORINATED BIPHENYLS BY MICRO-ORGANISMS (BIODEGRADATION OF POLYCHLORINATED BIPHENYLS)
557	3/28/1975	ACTIVATED SLUDGE PRIMARY BIODEGRADATION OF POLYCHLORINATED BIPHENYLS
558	5/1/1975	BIODEGRADABILITY OF MONOCHLOROBIPHENYL (MCS 1547) ANALYTICAL CHEMISTRY SPECIAL STUDY 74-9 Report 3968
559	5/1/1975	TISSUE RESIDUES FROM SUBACUTE ORAL FEEDING OF DIELECTRIC FLUIDS AND REPLACEMENT CANDIDATES ANALYTICAL CHEMISTRY SPECIAL STUDY 74-6 Report 3968
560	7/1/1975	POND EXPERIMENT (PCB IN FISH AND WATER); Ruabon
561	11/1/1975	DETERMINATION OF AROCLOR 1016 IN AIR AT ELECTRIC UTILITIES COMPANY LASALLE ILLINOIS SPECTROSCOPY S-75-SS-42 Report 3979
562	1/1/1976	FINAL REPORT ON ANALYTICAL CHEMISTRY INVESTIGATIONS - 1973 SPECIAL STUDIES ST. LOUIS RESEARCH REPORT NO. 3967 (PARTIAL REPORT CONTAINS ONLY THE INDEX, SECTION TITLES, INTRODUCTION AND SUMMARY) (see 73-26: 73-27)
563	1/1/1976	FINAL REPORT ON ANALYTICAL CHEMISTRY INVESTIGATIONS - 1974 SPECIAL STUDIES REPORT NO. 3968 (PARTIAL REPORT CONTAINS ONLY THE INDEX, SECTION TITLES, INTRODUCTION AND SUMMARY) (see 74-6; 74-9)
564	7/1/1976	BIODEGRADABILITY OF PROCESS FLUIDS AND RELATED MATERIALS ANALYTICAL CHEMISTRY SPECIAL STUDY AC-75-SS-15 Report 3991
565	7/1/1976	BIODEGRADABILITY OF DIELECTRIC FLUIDS AND COMPONENTS ANALYTICAL CHEMISTRY SPECIAL STUDY AC-75-SS-16 Report 3991
566	12/1/1976	METHOD FOR THE QUANTITATIVE DETERMINATION OF POLYCHLOROBIPHENYL IN WORKPLACE AIR S-76-M-24 Report 126
567	4/7/1978	WORK PLAN FOR THE ANALYSIS OF PCB CONTENT IN FAT TISSUE SPECIMENS
568	1/1/1980	PCB DEGRADATION: TRANSFORMATION BY ANAEROBIC BACTERIA FROM THE HUDSON RIVER
569	1/1/1980	FUNGAL DEGRADATION OF PCBS (ABSTRACT)
570	7/1/1980	DETERMINATION OF BIPHENYL (BP) POLYCHLORINATED BIPHENYLS (PCBS) AND HEXACHLOROBENZENE (HCB) IN LOW BOILING MATRICES REPORT ES-80-M-11 Report MSL 1567
571	9/19/1980	REPORT ES-80-SS-18 POLYCHLORINATED BIPHENYLS IN SAMPLES FROM CAULKS CREEK SITE Report MSL 1568
572	6/2/1905	MEASUREMENT OF SELECTED CHEMICALS IN SOIL FROM THE DEAD CREEK SITE ILLINOIS EPA SPLIT SAMPLES REPORT ES-80-SS-24 Report MSL 1568

Item	Date	Title*
573	6/2/1905	MEASUREMENT OF SELECTED CHEMICALS IN SOIL FROM THE DEAD CREEK SITE WG KRUMMRICH PLANT SAMPLINGS ES-80-SS-26 Report MSL 1568
574	6/2/1905	MEASUREMENT OF SELECTED CHEMICALS IN SOIL FROM THE DEAD CREEK SITE REPORT ES-80-SS-27 Report MSL 1567
575	10/1/1980	DETERMINATION OF POLYCHLORINATED BIPHENYLS (PCBS) IN SOIL AND SEDIMENT REPORT ES-80-M-28 Report MSL 1567
576	1/1/1981	DEGRADATION OF POLYCHLORINATED BIPHENYLS
577	2/27/1981	MEASUREMENT OF POLYCHLORINATED BIPHENYLS IN SOIL CORES FROM WG KRUMMRICH PLANT STUDY ES-81-SS-17 Report MSL 2327
578	8/27/1981	STATISTICAL ANALYSIS OF PCB CONTENT OF MONKEY TISSUE STUDY S-81-SS-2 Report MSL 2211
579	9/16/1982	ANALYSIS OF PORT PLASTICS SERUM SAMPLES FOR PCBS REPORT MDA-323
580	2/25/1983	PCB CONTENT OF MONKEY TISSUE REPORT NO. MDA-041

* List consists of analytical studies included in Monsanto list of studies (Plaintiff's Exhibit No. Kaley 20; 11-17-11)

3. Attachment 1C. Reviewed PCB-related product bulletins

- 1932 - Aroclors [PCB-ARCH0274831].pdf** - Physical Properties of The Aroclors. Aroclor #1242, Aroclor #1254, Aroclor #1262, Aroclor #1268, Aroclor #4465, Aroclor #2565 (PCB-ARCH0274831)
- 1934-07-17 - Aroclors [PCB-ARCH0207747].pdf** - Physical Properties of The Aroclors. Aroclor #1242, Aroclor #1248, Aroclor #1254, Aroclor #1262, Aroclor #1269, Aroclor #4465, Aroclor #2565 (PCB-ARCH0207747)
- 1934 or before - Aroclors-Lacquers [PCB-ARCH0222139].pdf** - The Properties of Two Aroclors of Special Interest to Manufacturers of Lacquers. Aroclors 1254 and 1262. (PCB-ARCH0222139)
- 1935-01-01 - Aroclors [PCB-ARCH0523839]** - Physical Properties of The Aroclors. Aroclor #1242, Aroclor #1248, Aroclor #1254, Aroclor #1262, Aroclor #1269, Aroclor #4465, Aroclor #2565 (PCB-ARCH0523839)
- 1940-05-01 - Plasticizers and Resins [PCB-ARCH0617319].pdf** - Plasticizers and Resins. Specifications and Application Data (PCB-ARCH0617319)
- 1943-04-00 - Aroclors [PCB-ARCH0616206].pdf** - The Aroclors. Physical Properties and Suggested Applications. Application Data Bulletin No. P-115 (PCB-ARCH0616206)
- 1946-03-01 - Plasticizers and Resins [PCB-ARCH0617258].pdf** - Plasticizers and Resins. Specifications and Application Data (PCB-ARCH0617258)
- 1946-01-01 - Aroclors [PCB-ARCH0232158].pdf** - The Aroclors. Physical Properties and Suggested Applications. Application Data Bulletin No. P-115 (PCB-ARCH0232158)
- 1947-08-01 - Aroclors [PCB-ARCH0232133].pdf** - The Aroclors. Physical Properties and Suggested Applications. Application Data Bulletin No. P-115 (PCB-ARCH0232133)
- 1948-11-01 - Co-Plasticizer-DOP-Vinyl Organosols and Pastes [PCB-ARCH0232353].pdf** - Aroclor 1254. Co-Plasticizer with DOP for Vinyl Organosols and Pastes. Monsanto Technical Bulletin No. P-134 (PCB-ARCH0232353)
- 1948-04-15 - Aroclor-High Pressure Compressors [PCB-ARCH0616283].pdf** - Aroclor. Incombustible Lubricants used in High-Pressure Compressors. Monsanto Technical Bulletin No. P-128 (PCB-ARCH0616283)
- 1948-05-01 - Co-Plasticizers-Polyvinylchloride [PCB-ARCH0390250].pdf** - Aroclors as Co-Plasticizers for Polyvinylchloride. Monsanto Technical Bulletin No. P-131 (PCB-ARCH0390250)
- 1948-01-01 - Aroclors-Chlorinated Rubber [PCB-ARCH0629491].pdf** - Aroclors as Used in Chlorinated Rubber. Monsanto Technical Bulletin No. P-124 (PCB-ARCH0629491)
- 1948-03-01 - Aroclors-Pliolite [PCB-ARCH0564354].pdf** - Aroclors as used in Pliolite S-5. Monsanto Technical Bulletin No. P-126 (PCB-ARCH0564354)
- 1948-06-01 - Carnauba Wax [PCB-ARCH0523261].pdf** - Aroclors as used to Extend or Substitute Carnauba Wax. Monsanto Technical Bulletin No. P-132. June 1, 1948 (PCB-ARCH0523261)
- 1948-09-01 - Carnauba Wax [PCB-ARCH0616000].pdf** - Aroclors as used to Extend or Substitute Carnauba Wax. Monsanto Technical Bulletin No. P-132. Sept. 1, 1948 (PCB-ARCH0616000)

- 1948-10-01 - Aroclors [PCB-ARCH0267230].pdf** - The Aroclors. Physical Properties and Suggested Applications. Application Data Bulletin No. P-115 (PCB-ARCH0267230)
- 1949-09-01 - Heat Transfer [PCB-ARCH0523245].pdf** - An Indirect Aroclor Heater for Unit Chemical Operations. Monsanto Technical Bulletin No. P-130 (PCB-ARCH0523245)
- 1949-04-04 - Lubricant-Plasticizer-Paper Draperies [PCB-ARCH0523777].pdf** - Aroclor 1254. Lubricant and Plasticizer in the Manufacture of Paper Draperies. Monsanto Technical Bulletin No. P-138 (PCB-ARCH0523777)
- 1949-03-28 - Hydraulic Fluid-Die-Casting Systems [PCB-ARCH0523806].pdf** - Aroclor. A Nonflammable Hydraulic Fluid for Die-Casting Systems. Monsanto Technical Bulletin No. P-137 (PCB-ARCH0523806)
- 1949-03-18 - Carnauba Wax [PCB-ARCH0232339].pdf** - Aroclors as used to Extend or Substitute Carnauba Wax. Monsanto Technical Bulletin No. P-132. March 18, 1949 [handwritten: supercedes Sept 1, 1948] (PCB-ARCH0232339)
- 1949-04-01 - Aroclors [PCB-ARCH0524127].pdf** - The Aroclors. Physical Properties and Suggested Applications. Application Data Bulletin No. P-115 (PCB-ARCH0524127)
- 1950-05-01 - Hydraulic Fluid-Die-Casting Systems [PCB-ARCH0090603].pdf** - Aroclor. A Nonflammable Hydraulic Fluid for Die-Casting Systems. Monsanto Technical Bulletin No. P-137 (PCB-ARCH0090603)
- 1950-06-01 - Aroclors [PCB-ARCH0522252].pdf** - The Aroclors. Physical Properties and Suggested Applications. Application Data Bulletin No. P-115 (PCB-ARCH0522252)
- 1952-11-01 - Aroclors [PCB-ARCH0498146].pdf** - The Aroclors. Physical Properties and Suggested Applications. Application Data Bulletin No. P-115 (PCB-ARCH0498146)
- 1952-01-01 - Aroclors [PCB-ARCH0524071].pdf** - The Aroclors. Physical Properties and Suggested Applications. Application Data Bulletin No. P-115 (PCB-ARCH0524071)
- 1953-10-01 - Co-Plasticizer-DOP-Vinyl Organosols and Pastes [PCB-ARCH0390194].pdf** - Aroclor 1254. Co-Plasticizer with DOP for Vinyl Organosols and Pastes. Monsanto Technical Bulletin No. P-134 (PCB-ARCH0390194)
- 1953-02-03 - Lindane [PCB-ARCH0231494].pdf** - Lindane-Aroclor Combinations. Monsanto Bulletin No. EX-43 (PCB-ARCH0231494)
- 1953-03-01 - Aroclors [PCB-ARCH0207716].pdf** - The Aroclors. Physical Properties and Suggested Applications. Application Data Bulletin No. P-115 (PCB-ARCH0207716)
- 1953-02-01 - Aroclors - High Styrene [PCB-ARCH0522892].pdf** - The Use of Aroclors in Formulating High Styrene Copolymer Surface Coatings (Marbon 9200). Monsanto Technical Bulletin No. P-149 (PCB-ARCH0522892)
- 1954-12-01 - Pydraul F9 (hydraulic) [PCB-ARCH0522346].pdf** - Pydraul F-9 (PCB-ARCH0522346)
- 1954-03-01 - Aroclors [PCB-ARCH0019006].pdf** - The Aroclors. Physical Properties and Suggested Applications. Application Data Bulletin No. O-P-115 (CB-ARCH0019006)

- 1954-12-01 - Proper Handling of Aroclors and Mixtures in Electrical Industry [PCB-ARCH0475346]** - The Proper Handling of Aroclors and Their Mixtures in the Electrical Industry, P.G. Benignus (PCB-ARCH0475346)
- 1955-10-01 - Heat Transfer [PCB-ARCH0390200].pdf** - An Indirect Aroclor Heater for Unit Chemical Operations. Monsanto Technical Bulletin No. O-130 (MONS076335, 0358627, PCB-ARCH0390200)
- 1955-11-01 - Plasticizer-Polyvinyl Acetate Emulsion Adhesives [PCB-ARCH0523798].pdf** - Aroclor 1221, 1232 and 1242 for Polyvinyl Acetate Emulsion Adhesives. Monsanto Technical Bulletin No. O-111 (PCB-ARCH0523798)
- 1955-01-01 - Heat Transfer [PCB-ARCH0390206].pdf** - Aroclor 1248. For Safe, Forced Circulation Heating to 600 F, Fire-Resistant Indirect Heating (PCB-ARCH0390206)
- 1956-04-01 - Heat Transfer [PCB-ARCH0090861].pdf** - Aroclor 1248. A Fire-Resistant, Heat-Transfer Agent that Operates up to 600 F in the Liquid Phase (PCB-ARCH0090861)
- 1956-01-01 - Plasticizers [PCB-ARCH0523505].pdf** - Product-Building for Profits with Monsanto Plasticizers. Information Booklet (PCB-ARCH0523505)
- 1956-10-01 - Pydraul AC (hydraulic) [PCB-ARCH0623142].pdf** - Pydraul AC Fire-Resistant Compressor Lubricant. Technical Bulletin O-133 (PCB-ARCH0623142)
- 1956-08-01 - Pydraul AC (hydraulic) [PCB-ARCH0617487].pdf** - Pydraul AC Fire-Resistant Compressor Lubricant. Technical Bulletin ODB-56-27 (PCB-ARCH0617487)
- 1956-11-01 - Aroclors [PCB-ARCH0298421].pdf** - The Aroclors. Physical Properties and Suggested Applications. Application Data Bulletin No. O-P-115 (PCB-ARCH0298421)
- 1956-05-01 - Proper Handling - Electrical Industry [PCB-ARCH0143101].pdf** - The Proper Handling of Aroclors and Their Mixtures in the Electrical Industry, P.G. Benignus (PCB-ARCH0143101)
- 1957-05-01 - Plasticizers-Chlorinated Rubber [PCB-ARCH0629121].pdf** - Aroclor Resins and Plasticizers for Chlorinated Rubber. Monsanto Technical Bulletin No. O-124 (PCB-ARCH0629121)
- 1957-07-01 - Pydraul AC (hydraulic) [PCB-ARCH0524856].pdf** - Pydraul AC Fire-Resistant Lubricant for Air Compressors (PCB-ARCH0524856)
- 1957-11-01 - Aroclors [PCB-ARCH0018976].pdf** - The Aroclors. Physical Properties and Suggested Applications. Application Data Bulletin No. O-P-115 (PCB-ARCH0018976)
- 1958-06-01 - Aroclor 1248 [PCB-ARCH0390276].pdf** - Aroclor 1248 (PCB-ARCH0390276)
- 1958-01-01 - Aroclor resins and plasticizers [PCB-ARCH0523693].pdf** - Aroclor Resins and Plasticizers for Chlorinated Rubber. Monsanto Technical Bulletin No. PL-311 (PCB-ARCH0523693)
- 1958-01-01 - Dielectric Fluids [PCB-ARCH0615744].pdf** - Monsanto Dielectric Fluids: Aroclors, Pyroclor (MONS079882, 0613372, PCB-ARCH0615744)
- 1959-07-01 - Aroclor 1221, 1232, 1242 [PCB-ARCH0306404].pdf** - Aroclor 1221, 1232 and 1242 for Polyvinyl Acetate-Emulsion Adhesives. Monsanto Technical Bulletin No. PL-321 (PCB-ARCH0306404)
- 1959-07-01 - Aroclor 1248 [PCB-ARCH0615831].pdf** - Aroclor 1248 (PCB-ARCH0615831)

- 1959-05-01 - Aroclor resins and plasticizers [PCB-ARCH0216209].pdf** - Aroclor Resins and Plasticizers for Chlorinated Rubber. Monsanto Technical Bulletin No. PL-311 (replaces O-124) (PCB-ARCH0216209)
- 1959-12-01 - Pasticizers in Synthetic Resin Adhesives [PCB-ARCH0524664]** - Plasticizers in Synthetic-Resin Adhesives. Technical Bulletin No. PL-307 (PCB-ARCH0524664)
- 1959-03-01 - Pydraul A-200 [PCB-ARCH0623418].pdf** - Pydraul A-200 (PCB-ARCH0623418)
- 1960-04-25 - Aroclor HDK-1 [PCB-ARCH0162800].pdf** - Aroclor HDK-1. Technical Data Sheet (PCB-ARCH0162800)
- 1960-12-01 - Aroclor Plasticizers [PCB-ARCH0266982].pdf** - Aroclor Plasticizers. Monsanto Technical Bulletin PL-306 (PCB-ARCH0266982)
- 1960-01-01 - Aroclors in Polyester & Epoxy Resins [PCB-ARCH0615972]** - Aroclors in Polyester and Epoxy Resins. Technical Service Bulletin G5/1 (PCB-ARCH0615972)
- 1960-06-01 - Bulk Receiving of Plasticizers [PCB-ARCH0232807]** - Bulk Receiving of Plasticizers. Technical Bulletin No. PL-314 (PCB-ARCH0232807)
- 1960-08-01 - Pydraul 150 [PCB-ARCH0092664].pdf** - Pydraul 150 (PCB-ARCH0092664)
- 1960-08-01 - High Viscosity Fire-Resistant Hydraulic Fluid for Heavy Duty App [PCB-ARCH0092668].pdf** - Pydraul 625 (PCB-ARCH0092668)
- 1960-08-01 - Pydraul F-9 [PCB-ARCH0092660].pdf** - Pydraul F-9 (PCB-ARCH0092660)
- 1960-05-01 - The Aroclor Compounds [PCB-ARCH0267128].pdf** - The Aroclor Compounds (PCB-ARCH0267128)
- 1960-01-01 - Proper Handling of Aroclors-Electric Indsutry [PCB-ARCH0553764].pdf** - The Proper Handling of Aroclors and Their Mixtures in the Electrical Industry, P.G. Benignus (PCB-ARCH0553764)
- 1962-01-01 - Aroclor 1248 [PCB-ARCH0544255].pdf** - Aroclor 1248 (PCB-ARCH0544255)
- 1962-04-01 - Aroclor Resins & Plasticizers for Chlorinated Rubber [PCB-ARCH0298332].pdf** - Aroclor Resins and Plasticizers for Chlorinated Rubber. Monsanto Technical Bulletin No. PL-311 (PCB-ARCH0298332)
- 1962-08-01 - Askarel Inspection & Maintenance Guide [PCB-ARCH0376014].pdf** - Askarel. Inspection and Maintenance Guide (PCB-ARCH0376014)
- 1962-05-01 - Askarel Transformer Fluid [PCB-ARCH0143013].pdf** - Care and Grooming of Askarel Transformer Fluid (PCB-ARCH0143013)
- 1962-12-01 - Therminol Fluid Heat Systems [PCB-ARCH0443533].pdf** - Therminol FR Fluid Heat Systems Engineering Heat Transfer Data. A Design Guide for Engineering Low-Cost, Low-Pressure Fire-Resistant Heat Transfer Systems (PCB-ARCH0443533)
- 1962-05-01 - Askarel-Type Transformers [PCB-ARCH0101643].pdf** - When You Want Fire Safety, Reliability, and Minimim Maintenance, Specify Askarel-Type Transformers (PCB-ARCH0101643)
- 1963-04-01 - Aroclor 1221, 1232, 1242 for Polyvinyl Acetate-Emulsion Adhesives [PCB-ARCH0018688].pdf** - Aroclor 1221, 1232 and 1242 for Polyvinyl Acetate-Emulsion Adhesives. Monsanto Technical Bulletin No. PL-321 (PCB-ARCH0018688)
- 1963-09-01 - Askarel Inspection & Maintenance Guide [PCB-ARCH0255907].pdf** - Askarel. Inspection and Maintenance Guide (PCB-ARCH0255907)

- 1964-01-01 - Pydraul Selector [PCB-ARCH0231313].pdf** - Pydraul Fire-Resistant Hydraulic Fluid Selector (PCB-ARCH0231313)
- 1965-08-01 - Askarel Inspection & Maintenance Guide [PCB-ARCH0143041].pdf** - Askarel. Inspection and Maintenance Guide (PCB-ARCH0143041)
- 1965-01-01 - Pydraul 312 [PCB-ARCH0622259].pdf** - Pydraul 312. Technical Bulletin (PCB-ARCH0622259)
- 1965-09-01 - Pydraul 312 [PCB-ARCH0443826].pdf** - Pydraul 312. Technical Bulletin P-312 [handwritten: obsolete 12/66] (PCB-ARCH0443826)
- 1965-08-01 - Therminol Fluid Heat Systems [PCB-ARCH0141010].pdf** - Therminol FR Fluid Heat Systems Engineering Heat Transfer Data. A Design Guide for Engineering Low-Cost, Low-Pressure Fire-Resistant Heat Transfer Systems (PCB-ARCH0141010)
- 1965-06-01 - Therminol Fluid Heat Systems [PCB-ARCH0443483].pdf** - Therminol FR Fluid Heat Systems Engineering Heat Transfer Data. A Design Guide for Engineering Low-Cost, Low-Pressure Fire-Resistant Heat Transfer Systems [hand-corrected copy dated June, 1965] (PCB-ARCH0443483)
- 1966-03-01 - Aroclor for Capacitors [PCB-ARCH0524735].pdf** - Aroclor for Capacitors (PCB-ARCH0524735)
- 1966-01-01 - Aroclor for Capacitors [PCB-ARCH0524773].pdf** - Aroclor for Capacitors (PCB-ARCH0524773)
- 1966-09-01 - Aroclor Plasticizers [PCB-ARCH0544340].pdf** - Aroclor Plasticizers. Monsanto Technical Bulletin O/PL-306 (PCB-ARCH0544340)
- 1966-10-31 - Aroclor Plasticizers [PCB-ARCH0216396].pdf** - Aroclor Plasticizers. Monsanto Technical Bulletin O/PL-306 [handwritten label states: Sample book. Revised 10-31-66] (PCB-ARCH0216396)
- 1966-01-01 - Pydraul 135 [PCB-ARCH0621777].pdf** - Pydraul 135. Fire-Resistant Hydraulic Fluid [hand-written O/FF-4] (PCB-ARCH0621777)
- 1966-09-01 - Pydraul 135 [PCB-ARCH0524943].pdf** - Pydraul 135. Fire-Resistant Hydraulic Fluid O/FF-4 (PCB-ARCH0524943)
- 1966-11-01 - Pydraul 312 [PCB-ARCH0617434].pdf** - Pydraul 312. Fire-Resistant Hydraulic Fluid. Technical Bulletin O/FF-7 (PCB-ARCH0617434)
- 1966-03-01 - Pydraul AC Fact Finder [PCB-ARCH0063692].pdf** - Pydraul AC Fire-Resistant Lubricant for Air Compressors. Fact Finder (PCB-ARCH0063692)
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- 1967-05-01 - Aroclor [PCB-ARCH0616105].pdf** - Aroclor Resins and Plasticizers for Chlorinated Rubber. Monsanto Technical Bulletin No. O/PL-311 (PCB-ARCH0616105)
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- 1967-08-01 - Askarel [PCB-ARCH0231169].pdf** - Here's How to Get More Performance and Save Money when Specifying Transformers [hand-written: Sample book, new 8/67] (PCB-ARCH0231169)
- 1967-04-01 - Pydraul 315 Fire Resistant Hydraulic Fluid [PCB-ARCH0617444].pdf** - Pydraul 135. Fire-Resistant Hydraulic Fluid O/FF-4 (EX) (PCB-ARCH0617444)
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- 1968-01-01 - Aroclor Plasticizers [PCB-ARCH0143389].pdf** - Aroclor Plasticizers. Monsanto Technical Bulletin O/PL-306 (PCB-ARCH0143389; PCB-ARCH0522411)
- 1968-05-01 - Aroclor Plasticizers [PCB-ARCH0568314].pdf** - Aroclor Plasticizers. Monsanto Technical Bulletin O/PL-306 (PCB-ARCH0568314)
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- 1968-10-01 - Pydraul Fire Resistant Hydraulic Fluid Selector 2 [PCB-ARCH0063592].pdf** - Pydraul Fire-Resistant Hydraulic Fluid Selector 2. O/FF-11 (PCB-ARCH0063592)
- 1968-09-01 - Pydraul Fire Resistant Hydraulic Fluid Selector 2 [PCB-ARCH0063609].pdf** - Pydraul Fire-Resistant Hydraulic Fluid Selector 2. O/FF-11 (PCB-ARCH0063609)
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- 1969-09-01 - Aroclor Plasticizers [PCB-ARCH0515425].pdf** - Aroclor Plasticizers. Monsanto Technical Bulletin O/PL-306A (PCB-ARCH0515425)

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- 1969-06-01 - Pydraul Fire Resistant Hydraulic Fluid Selector 1 [PCB-ARCH0524883].pdf** - Pydraul Fire-Resistant Hydraulic Fluid Selector 1. O/FF-3 (PCB-ARCH0524883)
- 1969-06-01 - Pydraul Fire Resistant Hydraulic Fluid Selector 2 [PCB-ARCH0522330].pdf** - Pydraul Fire-Resistant Hydraulic Fluid Selector 2. O/FF-11 (PCB-ARCH0522330)
- 1969-11-01 - Pyroclor [PCB-ARCH0024820].pdf** - Pyroclor 5. Transformer askarel by Monsanto. Technical Data Sheet (PCB-ARCH0024820)
- 1969-08-01 - Askarel Fire Resistant Transformers Offer [PCB-ARCH0524995].pdf** - When Specifying Transformers Here's How to Get More Performance and Save Money: Askarel the Fire-Resistant Dielectric Preferred by Specifiers Who Want to Save Money Now and for Years to Come (PCB-ARCH0524995)
- 1970-09-01 - Aroclor Plasticizers [PCB-ARCH0566489].pdf** - Aroclor Plasticizers (PCB-ARCH0566489)
- 1970-01-01 - Aroclor Plasticizers [PCB-ARCH0580282].pdf** - Aroclor Plasticizers (PCB-ARCH0580282)
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- 1970-07-01 - The Compressor Lubricant Fact Finder [PCB-ARCH0019158].pdf** - Pydraul Air Compressor Lubricants by Monsanto. The Compressor Lubricant Fact Finder. O/FF-2 (PCB-ARCH0019158)
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- 1970-02-01 - Standard Analytical Methods for Determining Therminol [PCB-ARCH0522708].pdf** - Standard Analytical Methods for Determining Therminol: Viscosity, Acidity, Moisture. Monsanto Technical Bulletin O/FF-18 (PCB-ARCH0522708)

- 1970-01-01 - Therminol Conversion [PCB-ARCH0588885].pdf** - Therminol Conversion Interim Bulletin SP-1TC (PCB-ARCH0588885)
- 1971-10-01 - Aroclor & Pyroclor [PCB-ARCH0277364]** - Aroclor & Pyroclor. Bulk Handling (PCB-ARCH0277364)
- 1971-04-01 - Aroclor [PCB-ARCH0232767].pdf** - Aroclor Resins and Plasticizers for Chlorinated Rubber. Monsanto Technical Bulletin No. O/PL-311 (PCB-ARCH0232767)
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- 1971-04-01 - Askarel Inspection and Maintenance Guide [PCB-ARCH0168693].pdf** - Askarel. Inspection and Maintenance Guide (PCB-ARCH0168693)
- 1971-06-01 - Askarel Fire Resistant Transformers Offer [PCB-ARCH0442397].pdf** - Here's How to Get More Performance and Save Money when Specifying Transformers (PCB-ARCH0442397)
- 1971-01-01 - Askarel Fire Resistant Transformers Offer [PCB-ARCH0616590].pdf** - Here's How to Get More Performance and Save Money when Specifying Transformers (PCB-ARCH0616590)
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- 1971-04-01 - Thermal Liquids A New Concept in Heating [PCB-ARCH0522672]** - Thermal Liquids: A New Concept in Heating (PCB-ARCH0522672)
- 1971-12-08 - Therminol Conversion Bulletin [PCB-ARCH0588615].pdf** - Therminol Conversion Bulletin (PCB-ARCH0588615)
- 1971-12-01 - Therminol Conversion Bulletin [PCB-ARCH0247276].pdf** - Therminol Conversion Bulletin SP/TC-1 (PCB-ARCH0247276)
- 1971-07-01 - Askarel Fire Resistant Transformers Offer [PCB-ARCH0460107].pdf** - When Specifying Transformers Here's How to Get More Performance and Save Money: Askarel Fire-Resistant Transformers Offer O/FF-14 (PCB-ARCH0460107)
- 1972-01-01 - Aroclor 1242 E1 [PCB-ARCH0523419].pdf** - Aroclor 1242 E1 (PCB-ARCH0523419)
- 1972-01-01 - Aroclor Plasticizers [PCB-ARCH0143469].pdf** - Aroclor Plasticizers. Monsanto Technical Bulletin O/PL-306A (PCB-ARCH0143469)
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- 1972-02-15 - Therminol Conversion Bulletin [PCB-ARCH0498321].pdf** - Therminol Conversion Bulletin SP/TC-1 (PCB-ARCH0498321)
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- 1973-08-01 - Aroclor [PCB-ARCH0036393]** - Aroclor (PCB-ARCH0036393)
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^no date - Aroclor for Capacitors [PCB-ARCH0231711].pdf - Aroclor for Capacitors (PCB-ARCH0231711)

^no date - Aroclor for Capacitors [PCB-ARCH0231737].pdf - Aroclor for Capacitors (PCB-ARCH0231737)

^no date - Aroclor Plasticizers [PCB-ARCH0530225].pdf - Aroclor Plasticizers (PCB-ARCH0530225)

^no date - Converting to Pydraul Hydraulic Fluids [PCB-ARCH0633434].pdf - Converting to Pydraul Hydraulic Fluids (PCB-ARCH0633434)

^no date - Plasticizers [PCB-ARCH0632796].pdf - Monsanto Plasticizers (PCB-ARCH0632796)

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B. Attachment 2. Treon studies listed in Shubik and Hartwell, 1957

148. --ETHYL ACRYLATE $\text{CH}_2=\text{CHCOOC}_2\text{H}_5$								
Treon et al., 1949c	1 rabbit	----	----	5 ml., 24 applications in one day	Skin	0	----	53 d.
	2 rabbits	----	----	3 or 5 ml., 3 applications daily for 2 d.	Skin	0	100%	3 mos.
	4 rabbits	----	----	74.8 p.p.m., 50 seven hr. exposures daily	Inhalation	0	100%	70 d.
	2 guinea pigs	----	----	74.8 p.p.m., 50 seven hr. exposures daily	Inhalation	0	100%	70 d.
	2 rats	----	----	74.8 p.p.m., 50 seven hr. exposures daily	Inhalation	0	100%	70 d.
	2 guinea pigs	----	----	26.2 p.p.m., 130 seven hr. exposures daily	Inhalation	0	100%	185 d.
	2 rats	----	----	26.2 p.p.m., 130 seven hr. exposures daily	Inhalation	0	100%	185 d.
	1 monkey	----	----	26.2 p.p.m., 130 seven hr. exposures daily	Inhalation	0	100%	185 d.
	4 rabbits	----	----	24.5 p.p.m., 130 seven hr. exposures daily	Inhalation	0	100%	185 d.
	2 guinea pigs	----	----	24.5 p.p.m., 130 seven hr. exposures daily	Inhalation	0	100%	185 d.
	2 rats	----	----	24.5 p.p.m., 130 seven hr. exposures daily	Inhalation	0	100%	185 d.
	1 monkey	----	----	24.5 p.p.m., 130 seven hr. exposures daily	Inhalation	0	100%	185 d.
	2 rabbits	----	----	0.0315 gm. per kg., 25 doses	P.O.	0	100%	35 d.
194. --2-NITROPROPANE $(\text{CH}_3)_2\text{CHNO}_2$								
Treon and Dutra, 1952	Monkey	----	----	83 p.p.m. Air, 130 seven hr. exposures	Inhalation	0	1 alive at 191 d.	191 d.
	Guinea pigs	----	----	83 p.p.m. air, 130 seven hr. exposures	Inhalation	0	2 alive at 191 d.	191 d.
	Rabbits	----	----	83 p.p.m. air, 130 seven hr. exposures	Inhalation	0	4 alive at 191 d.	191 d.
54 of the 1000				197 Pulmonary atelectasis, mean number 6.9 (17% in the untreated controls).				
194. --2-NITROPROPANE $(\text{CH}_3)_2\text{CHNO}_2$ --Continued								
Treon and Dutra, 1952	Rats	----	----	83 p.p.m. air, 130 seven hr. exposures	Inhalation	0	1 alive at 191 d.	191 d.
	Cats	----	----	83 p.p.m. air, 130 seven hr. exposures	Inhalation	0	2 alive at 191 d.	191 d.
	Guinea pigs	----	----	317 p.p.m. air, 130 seven hr. exposures	Inhalation	0	2 alive at 199 d.	199 d.
	Rabbits	----	----	317 p.p.m. air, 130 seven hr. exposures	Inhalation	0	1 alive at 199 d.	199 d.
	Rats	----	----	317 p.p.m. air, 130 seven hr. exposures	Inhalation	0	2 at 199 d.	199 d.
237 --TRICHLOROACETONITRILE CCl_3CN								
Treon et al., 1949b	2 monkeys	----	----	0.84 or 5.3 p.p.m. air, 130 seven hr. exposures	Inhalation	0	100%	26 wks.
	4 cats	----	----	0.84 or 5.3 p.p.m. air, 130 seven hr. exposures	Inhalation	0	100%	26 wks.
	5 guinea pigs	----	----	0.84, 5.3 or 13.5 p.p.m. air, 50 or 130 seven hr. exposures	Inhalation	0	100%	26 wks.
	9 rabbits	----	----	0.84, 5.3 or 13.5 p.p.m. air, 22 to 130 seven hr. exposures	Inhalation	0	100%	26 wks.
	1 rabbit	----	----	6 ml., 1 hr. contact daily for 16 days	Skin	0	----	2 mos.
	2 rabbits	----	----	0.035 gm. per kg. daily, 5 - 6 days per wk. in propylene glycol, 30 - 32 doses	P.O.	0	100%	2 mos.
370. --2-ETHYLHEXYL DIPHENYL PHOSPHATE $(\text{C}_6\text{H}_5\text{O})_2\text{P}-\text{OCH}_2\text{CH}(\text{C}_2\text{H}_5)(\text{CH}_2)_3\text{CH}_3$								
Treon et al., 1953	4 rabbits	----	----	9.4 ml. per kg. undiluted	Skin	0	----	2 mos.
	6 dogs	----	----	0.5 ml. in capsules daily, and then 0.6 - 1.5% in the diet or 1.6 - 2.5% in the diet	P.O.	0	----	26 mos.
	160 rats	Garworth and Albino	MZF	0.625 - 5.0% in the diet	P.O.	0.405	102 died	2 yrs.

405. --N-MONOMETHYLANILINE



Treon et al., 1949a	5 rabbits	----	----	0.10 - 0.39 g. per kg. for periods of 1 hr., 12 - 27 times	Skin	0	1 died after 12 applications	50 d.
	4 rabbits	----	----	0.024 g. per kg. as 10% (W/V) in propylene glycol, 5 d. per wk., 77 - 100 doses	P.O.	0	100% lived	Over 20 wks.
	7 rabbits	----	----	0.055 - 0.18 g. per kg., 10% W/V in propylene glycol	P.O.	0	1 died at 1 mo.	2 mos.
Treon et al., 1950	3 rats	----	----	7.6 p.p.m., 130 seven hr. exposures	Inhalation	0	1 died	Over 26 wks.
	2 rats	----	----	2.4 p.p.m., 130 seven hr. exposures	Inhalation	0	100% alive	Over 26 wks.
	2 rats	----	----	26.6 p.p.m., 58 seven hr. exposures	Inhalation	0	100% alive	Over 11 wks.
	2 rats	----	----	86 p.p.m., 50 seven hr. exposures	Inhalation	0	100% alive	Over 10 wks.
	4 rabbits	----	----	2.3 p.p.m., 130 seven hr. exposures	Inhalation	0	100% alive	Over 26 wks.
	7 rabbits	----	----	7.6 p.p.m., 130 seven hr. exposures	Inhalation	0	6 died	Over 26 wks.
	4 rabbits	----	----	26.6 p.p.m., 58 seven hr. exposures	Inhalation	0	2 died	Over 10 wks.
	6 rabbits	----	----	86 p.p.m., 50 seven hr. exposures	Inhalation	0	3 died	Over 10 wks.
	1 monkey	----	----	2.4 p.p.m., 130 seven hr. exposures	Inhalation	0	----	Over 26 wks.
	3 cats	----	----	26.6 p.p.m., 58 seven hr. exposures	Inhalation	0	2 died	Over 11 wks.
	5 cats	----	----	2.4 or 7.6 p.p.m., 130 seven hr. exposures	Inhalation	0	----	Over 26 wks.

456. --XYLIDINE⁴⁴⁶



Treon et al., 1949a	2 rabbits	----	----	0.14 ml. per kg., 7 d. per wk. for 8 wk.	P.O.	0	----	8 wks.
	6 rabbits	----	----	0.12 - 0.62 g. per kg.	P.O.	0	----	2 mos.
Treon et al., 1950	2 cats	----	----	7.8 p.p.m., 92 seven hr. exposures	Inhalation	0	100% alive	Over 18.5 wks.
	2 cats	----	----	17.4 p.p.m., 31 seven hr. exposures	Inhalation	0	Both died by 6.5 wks.	6.5 wks.
	2 rats	----	----	17.4 p.p.m., 7 seven hr. exposures	Inhalation	0	100% alive	Over 14 wks.
	5 rats	----	----	60 p.p.m., 50 seven hr. exposures	Inhalation	0	3 died	Over 10 wks.
	1 guinea pig	----	----	17.4 p.p.m., 70 seven hr. exposures	Inhalation	0	----	Over 14 wks.
	2 guinea pigs	----	----	50 p.p.m., 55 seven hr. exposures	Inhalation	0	1 died	Over 10 wks.
	2 guinea pigs	----	----	60 p.p.m., 50 seven hr. exposures	Inhalation	0	1 died	Over 10 wks.
	4 rats	----	----	132 - 142 p.p.m., 50 seven hr. exposures	Inhalation	0	2 died	Over 10 wks.
	2 guinea pigs	----	----	132 - 142 p.p.m., up to 25 seven hr. exposures	Inhalation	0	1 died at 2 wks.	5 wks.
	2 rabbits	----	----	17.4 p.p.m., 70 seven hr. exposures	Inhalation	0	100% alive	Over 14 wks.
	4 rabbits	----	----	50 p.p.m., 60 seven hr. exposures	Inhalation	0	100% alive	Over 12 wks.
	4 rabbits	----	----	60 p.p.m., 50 seven hr. exposures	Inhalation	0	1 died	Over 10 wks.
	6 rabbits	----	----	132 - 142 p.p.m., 50 seven hr. exposures	Inhalation	0	100% alive	Over 10 wks.
	1 monkey	----	----	7.8 p.p.m., 92 seven hr. exposures	Inhalation	0	----	Over 18.5 wks.
	12 rabbits	----	----	2 - 50% in iso-octane	Skin	0	Several died early	50 d.

⁴⁴⁶ Mixture of isomers.

758. --2-AMINOPYRIMIDINE



Treon et al., 1948	2 rabbits	----	----	0.07 mg. per litre as vapor in air, 7 hrs. daily for 60 d.	Inhalation	0	100%	175 d.
	2 guinea pigs	----	----	0.07 mg. per litre as vapor in air, 7 hrs. daily for 60 d.	Inhalation	0	100%	175 d.

C. Attachment 3. Animal studies included in evaluation of potential immune effects of PCB

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Category	Endpoint	Species (sex)	Test article Dose levels	Dosing parameters	NOAEL dose	LOAEL dose	Notes for NOAEL and/or LOAEL	Reference
Cell mediated immunity	Cytotoxic T Lymphocyte assay: alloantigenic cells	Mouse (males)	Aroclor 1254 (– CDBFs) [140 mg/kg-d; estimated]	Route: ip Duration: 1/w; 4 w	-	14 mg/kg-d	-	(Clark <i>et al.</i> , 1983)
Cell mediated immunity	Cytotoxic T Lymphocyte assay: alloantigenic cells	Mouse (males)	PCB155 [20 mg/kg-d; estimated]	Route: ip Duration: 1/w; 6 w	20 mg/kg-d	-	-	(Clark <i>et al.</i> , 1983)
Cell mediated immunity	Cytotoxic T Lymphocyte assay: alloantigenic cells	Mouse (males)	PCB77 [2 mg/kg-d; estimated]	Route: ip Duration: 1/w; 5 w	-	2 mg/kg-d	LOAEL for ↓CTL = not specified beyond observation it occurred. Interpolated from graph at ~2 md/kg-d	(Clark <i>et al.</i> , 1983)
Cell mediated immunity	Cytotoxic T Lymphocyte assay: alloantigenic cells	Rat (males)	Aroclor 1254 0.1 to 25 mg/kg-d	Route: oral (gavage) Duration: 1/d; 15w	25 mg/kg-d	-	-	(Smialowicz <i>et al.</i> , 1989)
Cell mediated immunity	Cytotoxic T Lymphocyte assay: P815	Mouse (not specified)	Aroclor 1016 [30 mg/kg-d; estimated]	Route: oral (diet) Duration: 1/d; 40 w	30 mg/kg-d	-	-	(Silkworth and Loose, 1981)

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Category	Endpoint	Species (sex)	Test article Dose levels	Dosing parameters	NOAEL dose	LOAEL dose	Notes for NOAEL and/or LOAEL	Reference
Cell mediated immunity	Cytotoxic T Lymphocyte assay: P815	Mouse (males)	PCB153 30 to 300 mg/kg-d	Route: oral (gavage) Duration: single dose; sac 10 d after P815 injection	300 mg/kg-d	-	-	(Kerkvliet <i>et al.</i> , 1990)
Cell mediated immunity	Cytotoxic T Lymphocyte assay: P815	Mouse (males)	PCB156 10 to 100 mg/kg-d	Route: oral (gavage) Duration: single dose; sac 10 d after P815 injection	30 mg/kg-d	100 mg/kg-d	-	(Kerkvliet <i>et al.</i> , 1990)
Cell mediated immunity	Cytotoxic T Lymphocyte assay: P815	Mouse (males)	PCB169 1 to 10 mg/kg-d	Route: oral (gavage) Duration: single dose; sac 10 d after P815 injection	1 mg/kg-d	3 mg/kg-d	-	(Kerkvliet <i>et al.</i> , 1990)
Cell mediated immunity	Cytotoxic T Lymphocyte assay: P815	Mouse (males)	PCB169 1 to 100 mg/kg-d	Route: oral (gavage) Duration: single dose; sac 12 d after P815	5 mg/kg-d	10 mg/kg-d	-	(Kerkvliet and Baecher-Steppan, 1988)
Cell mediated immunity	Cytotoxic T Lymphocyte assay: P815	Mouse (females)	PCB169 1 to 100 mg/kg-d	Route: oral (gavage) Duration: single dose; sac 12 d after P815	5 mg/kg-d	10 mg/kg-d	-	(Kerkvliet and Baecher-Steppan, 1988)
Cell mediated immunity	Cytotoxic T Lymphocyte assay: P815	Mouse (males)	PCB169 10 or 100 mg/kg-d	Route: oral (gavage) Duration: single dose; sac 10 d after P815 injection	100 mg/kg-d	-	-	(Kerkvliet <i>et al.</i> , 1990)
Cell mediated immunity	Cytotoxic T Lymphocyte assay: P815	Mouse (male)	PCB169 10 mg/kg-d	Route: oral (gavage) Duration: single dose; 10 d post exposure	-	10 mg/kg-d	-	(De Krey <i>et al.</i> , 1993)

Category	Endpoint	Species (sex)	Test article Dose levels	Dosing parameters	NOAEL dose	LOAEL dose	Notes for NOAEL and/or LOAEL	Reference
417	Cell mediated immunity	Cytotoxic T Lymphocyte assay: P815	Mouse (males)	PCB169 10 mg/kg-d	Route: oral (gavage) Duration: single dose; sac 10 d after P815 injection	- 10 mg/kg-d	-	(Kerkvliet <i>et al.</i> , 1990)
	Cell mediated immunity	Cytotoxic T Lymphocyte assay: P815	Mouse (male)	PCB169 10 mg/kg-d	Route: oral (gavage) Duration: single dose; sac 9d post P815 injection	- 10 mg/kg-d	-	(De Krey <i>et al.</i> , 1994a)
	Cell mediated immunity	Cytotoxic T Lymphocyte assay: P815	Mouse (both)	PCB169 10 mg/kg-d	Route: oral (gavage) Duration: single dose; sac 10 d post exposure	- 10 mg/kg-d	-	(De Krey <i>et al.</i> , 1994b)
	Cell mediated immunity	Cytotoxic T Lymphocyte assay: P815	Mouse (both)	PCB169 10 mg/kg-d	Route: oral (gavage) Duration: single dose; sac 10-12 d after P815	- 10 mg/kg-d	-	(Kerkvliet and Baecher-Steppan, 1988)
	Cell mediated immunity	Delayed type hypersensitivity: OVA, ear thickness	Rat (males)	Mix (derived from native herring oil) Pups cumulative intake at 11d (2.03, 19.9 ng TEQ/kg Atlantic, Baltic oils); at 25 d (1.83, 17.3 ng TEQ/kg Atlantic, Baltic oils) mg/kg-d	Route: oral (gavage) Duration: 1/d; GD6 to pup weaning (41 exposure days)	- -	No difference in Baltic or Atlantic groups	(Ross <i>et al.</i> , 1997)

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Category	Endpoint	Species (sex)	Test article Dose levels	Dosing parameters	NOAEL dose	LOAEL dose	Notes for NOAEL and/or LOAEL	Reference
Cell mediated immunity	Delayed-type hypersensitivity response: Dinitrofluorobenzene [DNFB], skin thickness	Rabbit (both)	Aroclor 1248 [0.5, 4.0, or 13 mg/kg-d; estimated]	Route: oral (diet) Duration: 1/d; mated after 4w on diet, exposure continued through 4w of nursing when infants weaned and placed on normal diet. Tested @ 7w of age	4 mg/kg-d	13 mg/kg-d	-	(Thomas and Hinsdill, 1980)
Cell mediated immunity	Delayed-type hypersensitivity response: OVA, ear thickness	Guinea pig (females)	Clophen A60 [6 or 30 mg/kg-d; estimated]	Route: oral (diet) Duration: 1/d; 7 w	-	6 mg/kg-d	-	(Vos and van Driel-Grootenhuys, 1972)
Cell mediated immunity	Delayed-type hypersensitivity response: Oxazolone, ear proliferation	Mouse (both)	Aroclor 1254 0.167, 16.7 or 41.7 mg/kg-d	Route: oral (diet) Duration: 1/d; to 8 w of age	41.7 mg/kg-d	-	-	(Talcott and Koller, 1983)
Cell mediated immunity	Delayed-type hypersensitivity response: Tuberculin, skin sensitivity	Rabbit (males)	Aroclor 1254 0.18 to 6.54 mg/kg-d	Route: oral (diet) Duration: 1/d; 8 w	6.54 mg/kg-d	-	-	(Street and Sharma, 1975)
Cell mediated immunity	Graft-vs-host response: -	Mouse (not specified)	Aroclor 1016 [30 mg/kg-d; estimated]	Route: oral (diet) Duration: 1/d; 37 w	30 mg/kg-d	-	-	(Silkworth and Loose, 1981)
Cell mediated immunity	Graft-vs-host response: -	Mouse (males)	Aroclor 1016 [30 mg/kg-d; estimated]	Route: oral (diet) Duration: 1/d; Spleen index assessed 9d after inoculation	30 mg/kg-d	-	-	(Silkworth and Loose, 1979a)

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Category	Endpoint	Species (sex)	Test article Dose levels	Dosing parameters	NOAEL dose	LOAEL dose	Notes for NOAEL and/or LOAEL	Reference
Cell mediated immunity	Lymphocyte blastogenesis, T cell mitogen(s): ConA	Mouse (pups (both))	Aroclor 1242 [21 mg/kg-d; estimated]	Route: sc Duration: dams (3/wk); dams (2 w)	21 mg/kg-d	-	-	(Arena <i>et al.</i> , 2003)
Cell mediated immunity	Lymphocyte blastogenesis, T cell mitogen(s): ConA	Mouse (pups (both))	Aroclor 1254 [21 mg/kg-d; estimated]	Route: sc Duration: dams (3/wk); dams (2 w)	21 mg/kg-d	-	-	(Arena <i>et al.</i> , 2003)
Cell mediated immunity	Lymphocyte blastogenesis, T cell mitogen(s): ConA	Mouse (male)	Aroclor 1254 63 to 550 mg/kg-d	Route: ip Duration: single dose; injection 1 w before sac	550 mg/kg-d	-	-	(Wierda <i>et al.</i> , 1981)
Cell mediated immunity	Lymphocyte blastogenesis, T cell mitogen(s): ConA	Mouse (not specified)	Aroclor 1254 500 or 1000 mg/kg-d	Route: ip Duration: single dose; sac 4, 8 or 15 d post-PCB treatment	1000 mg/kg-d	-	-	(Lubet <i>et al.</i> , 1986)
Cell mediated immunity	Lymphocyte blastogenesis, T cell mitogen(s): ConA	Mouse (both)	Fenclor 42 500 or 1,000 mg/kg-d	Route: ip Duration: 1/d; single dose	-	500 mg/kg-d	-	(Franco <i>et al.</i> , 1989)
Cell mediated immunity	Lymphocyte blastogenesis, T cell mitogen(s): ConA	Mouse (both)	Fenclor 42 500 or 1,000 mg/kg-d	Route: ip Duration: 1/d; 15d	-	500 mg/kg-d	-	(Franco <i>et al.</i> , 1989)
Cell mediated immunity	Lymphocyte blastogenesis, T cell mitogen(s): ConA	Mouse (both (pooled))	Mix (Aroclor 1242:Aroclor 1254 2:1) [4.7 mg/kg-d; estimated]	Route: oral (diet) Duration: 1/d; to 22 w of age	-	4.7 mg/kg-d	-	(Segre <i>et al.</i> , 2002)

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Category	Endpoint	Species (sex)	Test article Dose levels	Dosing parameters	NOAEL dose	LOAEL dose	Notes for NOAEL and/or LOAEL	Reference
Cell mediated immunity	Lymphocyte blastogenesis, T cell mitogen(s): ConA	Mouse (females)	PCB118 82 to 260 mg/kg-d	Route: oral (gavage) Duration: single dose; sac 2 d after treatment	260 mg/kg-d	-	-	(Dahlman <i>et al.</i> , 1994)
Cell mediated immunity	Lymphocyte blastogenesis, T cell mitogen(s): ConA	Mouse (males)	PCB153 0.5 or 50 mg/kg-d	Route: ip Duration: every other day; 10 days (5 total injections)	0.5 mg/kg-d	50 mg/kg-d	-	(Kuiper <i>et al.</i> , 2016)
Cell mediated immunity	Lymphocyte blastogenesis, T cell mitogen(s): ConA	Mouse (females)	PCB153 0.125 or 12.5 mg/kg-d	Route: ip Duration: bi-weekly; up to 16 w (or until mouse became diabetic)	-	0.125 mg/kg-d	-	(Kuiper <i>et al.</i> , 2016)
Cell mediated immunity	Lymphocyte blastogenesis, T cell mitogen(s): ConA	Mouse (males)	PCB153 0.00009 to 1.4 mg/kg-d	Route: oral (diet) Duration: 1/d; 2w prior to mating through PND21; offspring followed for 1 yr	-	-	Analysis limited to trend analysis. No dose/response observed.	(van Esterik <i>et al.</i> , 2015)
Cell mediated immunity	Lymphocyte blastogenesis, T cell mitogen(s): ConA	Mouse (females)	PCB153 0.00009 to 1.4 mg/kg-d	Route: oral (diet) Duration: 1/d; 2w prior to mating through PND21; offspring followed for 1 yr	-	-	Analysis limited to trend analysis. No dose/response observed.	(van Esterik <i>et al.</i> , 2015)
Cell mediated immunity	Lymphocyte blastogenesis, T cell mitogen(s): ConA	Mouse (females)	PCB77 7.3 to 29 mg/kg-d	Route: oral (gavage) Duration: single dose; sac 2 d after treatment	29 mg/kg-d	-	-	(Dahlman <i>et al.</i> , 1994)

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Category	Endpoint	Species (sex)	Test article Dose levels	Dosing parameters	NOAEL dose	LOAEL dose	Notes for NOAEL and/or LOAEL	Reference
Cell mediated immunity	Lymphocyte blastogenesis, T cell mitogen(s): ConA	Rat (males)	Aroclor 1254 0.1 to 25 md/kg-d	Route: oral (gavage) Duration: 1/d; 15w	25 mg/kg-d	-	-	(Smialowicz <i>et al.</i> , 1989)
Cell mediated immunity	Lymphocyte blastogenesis, T cell mitogen(s): ConA, PHA	Goat (both)	PCB126 Maternal: 0.000049 mg/kg-d	Route: in utero and nursing (GD60 through lactation) Duration: 3/w; sac at 9 mo of age	0.000049 mg/kg-d	-	-	(Lyche <i>et al.</i> , 2004)
Cell mediated immunity	Lymphocyte blastogenesis, T cell mitogen(s): ConA, PHA	Goat (both)	PCB153 Maternal: 0.098 mg/kg-d	Route: in utero and nursing (GD60 through lactation) Duration: 3/w; sac at 9 mo of age	-	0.098 mg/kg-d	-	(Lyche <i>et al.</i> , 2004)
Cell mediated immunity	Lymphocyte blastogenesis, T cell mitogen(s): ConA, PHA	Monkey (females)	Aroclor 1254 0.005 to 0.08 mg/kg-d	Route: oral (gelatin capsule) Duration: 1/d; 55 mo	0.08 mg/kg-d	-	-	(Tryphonas <i>et al.</i> , 1991a)
Cell mediated immunity	Lymphocyte blastogenesis, T cell mitogen(s): ConA, PHA	Monkey (females)	Aroclor 1254 0.005 to 0.08 mg/kg-d	Route: oral (gelatin capsule) Duration: 1/d; 23 mo	0.08 mg/kg-d	-	-	(Tryphonas <i>et al.</i> , 1989)
Cell mediated immunity	Lymphocyte blastogenesis, T cell mitogen(s): ConA, PHA	Monkey (both)	Mix (major PCBs in Canadian human milk) 0.0075 mg/kg-d	Route: oral (in corn oil) Duration: 1/d; 20 wks exposure, 66 wks total (w/ follow-up)	0.0075 mg/kg-d	-	-	(Arnold <i>et al.</i> , 1999)
Cell mediated immunity	Lymphocyte blastogenesis, T cell mitogen(s): ConA, PHA	Mouse (not specified)	Aroclor 1016 [30 mg/kg-d; estimated]	Route: oral (diet) Duration: 1/d; up to 41w	-	30 mg/kg-d	-	(Silkworth and Loose, 1979b)

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Category	Endpoint	Species (sex)	Test article Dose levels	Dosing parameters	NOAEL dose	LOAEL dose	Notes for NOAEL and/or LOAEL	Reference
Cell mediated immunity	Lymphocyte blastogenesis, T cell mitogen(s): ConA, PHA	Mouse (dams exposed, both sex of pups)	Aroclor 1254 300 mg/kg-d	Route: in utero (ip to dams prior to mating) Duration: single dose; in utero/lactation exposure. Pups weaned at 22 d of age. Sac at 6 w of age.	-	300 mg/kg-d	-	(Wu <i>et al.</i> , 1999)
Cell mediated immunity	Lymphocyte blastogenesis, T cell mitogen(s): ConA, PHA	Mouse (dams exposed, both sex of pups)	Aroclor 1254 300 mg/kg-d	Route: in utero (ip to dams prior to mating) Duration: single dose; in utero/lactation exposure. Pups not weaned. Sac at 4 w of age.	300 mg/kg-d	-	-	(Wu <i>et al.</i> , 1999)
Cell mediated immunity	Lymphocyte blastogenesis, T cell mitogen(s): ConA, PHA	Rabbit (both)	Aroclor 1248 [0.5, 4.0, or 13 mg/kg-d; estimated]	Route: oral (diet) Duration: 1/d; mated after 4w on diet, exposure continued through 4w of nursing when infants weaned and placed on normal diet. Tested @ 7w of age	13 mg/kg-d	-	-	(Thomas and Hinsdill, 1980)
Cell mediated immunity	Lymphocyte blastogenesis, T cell mitogen(s): PHA	Mouse (male)	Aroclor 1016 [30 mg/kg-d; estimated]	Route: oral (diet) Duration: 1/d; 40 w	30 mg/kg-d	-	-	(Silkworth and Loose, 1981)
Cell mediated immunity	Lymphocyte blastogenesis, T cell mitogen(s): PHA	Mouse (males)	Aroclor 1016 [30 mg/kg-d; estimated]	Route: oral (diet) Duration: 1/d; 41w	30 mg/kg-d	-	-	(Silkworth and Loose, 1979a)

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Category	Endpoint	Species (sex)	Test article Dose levels	Dosing parameters	NOAEL dose	LOAEL dose	Notes for NOAEL and/or LOAEL	Reference
Cell mediated immunity	Lymphocyte blastogenesis, T cell mitogen(s): PHA	Rat (males)	Aroclor 1254 0.1 to 25 mg/kg-d	Route: oral (gavage) Duration: 1/d; 15w	10 mg/kg-d	25 mg/kg-d	-	(Smialowicz <i>et al.</i> , 1989)
Cell mediated immunity	Lymphocyte blastogenesis, T cell mitogen(s): PHA	Rat (males)	Aroclor 1254 [22 mg/kg-d; estimated]	Route: oral (diet) Duration: 1/d; 7 d	-	22 mg/kg-d	-	(Bonnyns and Bastomsky, 1976)
Cell mediated immunity	Lymphocyte blastogenesis, T cell mitogen(s): STM	Mouse (not specified)	Aroclor 1254 500 or 1000 mg/kg-d	Route: ip Duration: single dose; sac 4, 8 or 15 d post-PCB treatment	1000 mg/kg-d	-	-	(Lubet <i>et al.</i> , 1986)
Cell mediated immunity	Lymphocyte blastogenesis: ConA, PHA	Rat (females)	Mix (derived from native herring oil) Pups cumulative intake at 11d (2.03, 19.9 ng TEQ/kg Atlantic, Baltic oils); at 25 d (1.83, 17.3 ng TEQ/kg Atlantic, Baltic oils) mg/kg-d	Route: oral (gavage) Duration: 1/d; GD6 to pup weaning (41 exposure days)	-	-	-	(Ross <i>et al.</i> , 1997)
Cell mediated immunity	Lymphocyte blastogenesis: ConA, PHA	Rat (females)	Mix (derived from native herring) Atlantic: 0.3 ngTEQ/kg; Baltic: 1.6 ngTEQ/kg mg/kg-d	Route: oral (diet) Duration: 1/d; 4.5 mo	-	-	-	(Ross <i>et al.</i> , 1996)

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Category	Endpoint	Species (sex)	Test article Dose levels	Dosing parameters	NOAEL dose	LOAEL dose	Notes for NOAEL and/or LOAEL	Reference
Cell mediated immunity	Mixed Lymphocyte reaction: lymph node lymphocytes	Rat (males)	Aroclor 1254 0.1 to 25 mg/kg-d	Route: oral (gavage) Duration: 1/d; 15w	25 mg/kg-d	-	-	(Smialowicz <i>et al.</i> , 1989)
Cell mediated immunity	Mixed Lymphocyte reaction: peripheral blood mononuclear cells	Monkey (females)	Aroclor 1254 0.005 to 0.08 mg/kg-d	Route: oral (gelatin capsule) Duration: 1/d; 55 mo	0.08 mg/kg-d	-	-	(Tryphonas <i>et al.</i> , 1991a).
Cell mediated immunity	Mixed Lymphocyte reaction: splenocytes	Mouse (male)	Aroclor 1016 [30 mg/kg-d; estimated]	Route: oral (diet) Duration: 1/d; 40 w	30 mg/kg-d	-	-	(Silkworth and Loose, 1981)
Cell mediated immunity	Mixed Lymphocyte reaction: splenocytes	Mouse (males)	Aroclor 1016 [30 mg/kg-d; estimated]	Route: oral (diet) Duration: 1/d; 24 w	-	30 mg/kg-d	-	(Silkworth and Loose, 1979a)
Cell subset distributions	BAL cellularity	Rat (males)	Aroclor 1242 [0.75 mg/kg-d; estimated]	Route: inhalation Duration: up to 10 d; 2 h/d	0.75 mg/kg-d	-	-	(Hu <i>et al.</i> , 2010)
Cell subset distributions	BAL cellularity	Rat (females)	Mix (Aroclor 1242:Aroclor 1254 65:35) [0.033 mg/kg-d; estimated]	Route: inhalation Duration: 4 w; 2 h/d (w1), 1.5 hr/d (w2-4)	0.033 mg/kg-d	-	-	(Hu <i>et al.</i> , 2012)
Cell subset distributions	Circulating cells	Monkey (females)	Aroclor 1254 0.005 to 0.08 mg/kg-d	Route: oral (gelatin capsule) Duration: 1/d; 55 mo	0.08 mg/kg-d	-	-	(Tryphonas <i>et al.</i> , 1991a)

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Category	Endpoint	Species (sex)	Test article Dose levels	Dosing parameters	NOAEL dose	LOAEL dose	Notes for NOAEL and/or LOAEL	Reference
Cell subset distributions	Circulating cells	Monkey (females)	Aroclor 1254 0.005 to 0.08 mg/kg-d	Route: oral (gelatin capsule) Duration: 1/d; 23 mo	-	0.08 mg/kg-d	-	(Tryphonas <i>et al.</i> , 1989)
Cell subset distributions	Circulating cells	Monkey (both)	Mix (major PCBs in Canadian human milk) 0.0075 mg/kg-d	Route: oral (formula) Duration: 1/d; 20 wks exposure, 66 wks total (w/ follow-up)	0.0075 mg/kg-d	-	-	(Arnold <i>et al.</i> , 1999)
Cell subset distributions	Circulating cells	Monkey (males)	Mix (major PCBs in Canadian human milk) 0.0075 mg/kg-d	Route: oral (in corn oil) Duration: 1/d; 20 wks exposure, 66 wks total (w/ follow-up)	0.0075 mg/kg-d	-	-	(Arnold <i>et al.</i> , 1999)
Cell subset distributions	Circulating cells	Mouse (both)	PCB126 0.0005 to 0.5 mg/kg-d	Route: oral (gavage) Duration: 1/d; up to 7 d	-	0.0005 mg/kg-d	-	(Du <i>et al.</i> , 2019)
Cell subset distributions	Circulating cells	Rat (males)	PCB126 0.0001 mg/kg-d	Route: intranasal Duration: 1/d; 1/d for 15d	-	0.001 mg/kg-d	-	(Shimada <i>et al.</i> , 2015)
Cell subset distributions	Circulating cells	Rat (males)	PCB180 0.01 mg/kg-d	Route: intranasal Duration: 1/d; 1/d for 15d	0.01 mg/kg-d	-	-	(Shimada <i>et al.</i> , 2015)
Cell subset distributions	Spleen cellularity	Mouse (females)	PCB153 0.125 or 12.5 mg/kg-d	Route: ip Duration: bi-weekly; up to 16 w (or until mouse became diabetic)	-	0.125 mg/kg-d	-	(Kuiper <i>et al.</i> , 2016)

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Category	Endpoint	Species (sex)	Test article Dose levels	Dosing parameters	NOAEL dose	LOAEL dose	Notes for NOAEL and/or LOAEL	Reference
Cell subset distributions	Spleen cellularity	Mouse (males)	PCB153 0.5 or 50 mg/kg-d	Route: ip Duration: every other day; 10 days (5 total injections)	-	0.5 mg/kg-d	-	(Kuiper <i>et al.</i> , 2016)
Cell subset distributions	Thymus and spleen cellularity	Rat (females)	Mix (derived from native herring oil) Pups cumulative intake at 11d (2.03, 19.9 ng TEQ/kg Atlantic, Baltic oils); at 25 d (1.83, 17.3 ng TEQ/kg Atlantic, Baltic oils) mg/kg-d	Route: oral (gavage) Duration: 1/d; GD6 to pup weaning (41 exposure days)	-	-	-	(Ross <i>et al.</i> , 1997)
Cell subset distributions	Thymus and spleen cellularity	Rat (females)	Mix (derived from native herring) Atlantic: 0.3 ngTEQ/kg; Baltic: 1.6 ngTEQ/kg mg/kg-d	Route: oral (diet) Duration: 1/d; 4.5 mo	-	-	-	(Ross <i>et al.</i> , 1996)
Circulating factors	↑alpha1-thymosin	Monkey (female)	Aroclor 1254 0.005 to 0.08 mg/kg-d	Route: oral (gelatin capsule) Duration: 1/d; 55 mo	-	0.005 mg/kg-d	-	(Tryphonas <i>et al.</i> , 1991b)

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Category	Endpoint	Species (sex)	Test article Dose levels	Dosing parameters	NOAEL dose	LOAEL dose	Notes for NOAEL and/or LOAEL	Reference
Circulating factors	Corticosterone	Rat (male)	Aroclor 1254 0.1 to 25 mg/kg-d	Route: oral (gavage) Duration: 1/d; 15w	-	0.1 mg/kg-d	With No Stress: (Miller <i>et al.</i> , 1993) LOAEL (↑ serum corticosterone) =0.1 mg/kg; With Stress: NOAEL (changes in serum corticosterone) =25 mg/kg;	
Circulating factors	Hydrocortisone	Monkey (females)	Aroclor 1254 0.005 to 0.08 mg/kg-d	Route: oral (gelatin capsule) Duration: 1/d; 55 mo	0.08 mg/kg-d	-	-	(Tryphonas <i>et al.</i> , 1991a)
Circulating factors	Hydrocortisone	Monkey (females)	Aroclor 1254 0.005 to 0.08 mg/kg-d	Route: oral (gelatin capsule) Duration: 1/d; 23 mo	0.08 mg/kg-d	-	-	(Tryphonas <i>et al.</i> , 1989)
Circulating factors	Hydrocortisone	Monkey (females)	Aroclor 1254 0.005 to 0.08 mg/kg-d	Route: oral (gelatin capsule) Duration: 1/d; 22 mo	0.08 mg/kg-d	-	-	(Loo <i>et al.</i> , 1989)
Circulating factors	IL-2	Mouse (pups (both))	Aroclor 1242 [21 mg/kg-d; estimated]	Route: sc Duration: dams (3/wk); dams (2 w)	-	21 mg/kg-d	-	(Arena <i>et al.</i> , 2003)
Circulating factors	IL-2	Mouse (pups (both))	Aroclor 1254 [21 mg/kg-d; estimated]	Route: sc Duration: dams (3/wk); dams (2 w)	-	21 mg/kg-d	-	(Arena <i>et al.</i> , 2003)

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Category	Endpoint	Species (sex)	Test article Dose levels	Dosing parameters	NOAEL dose	LOAEL dose	Notes for NOAEL and/or LOAEL	Reference
Circulating factors	Circulating TNF- α , IFN- γ , IL-2	Mouse (both)	PCB126 0.0005 to 0.5 mg/kg-d	Route: oral (gavage) Duration: 1/d; up to 7 d	n/a, 0.0005, 0.0005 mg/kg for IFN- α , IFN- γ , IL-2, respectively	0.0005, 0.005, 0.005 mg/kg (for IFN- α , IFN- γ , IL-2, respectively)	-	(Du <i>et al.</i> , 2019)
Circulating factors	Serum fibronectin	Mouse (males)	Aroclor 1242 [0.9 or 18 mg/kg-d; estimated]	Route: oral (diet) Duration: 1/d; 18w	-	0.9 mg/kg-d	-	(Loose <i>et al.</i> , 1981)
Humoral immunity	Circulating IgG (radial immunodiffusion)	Rabbit (males)	Aroclor 1221 [15 mg/kg-d; estimated]	Route: oral (drinking water) Duration: 1/d; 3 mo	-	15 mg/kg-d	-	(Wasserman <i>et al.</i> , 1973)
Humoral immunity	Circulating IgG response (KLH antigen; ELISA)	Mouse (dams exposed, both sex of pups)	Aroclor 1254 300 mg/kg-d	Route: in utero (ip to dams prior to mating) Duration: single dose; in utero/lactation exposure. Pups weaned at 22 d of age. Sac at 6 w of age.	300 mg/kg-d	-	-	(Wu <i>et al.</i> , 1999)
Humoral immunity	Circulating IgG response (KLH antigen; ELISA)	Mouse (dams exposed, both sex of pups)	Aroclor 1254 300 mg/kg-d	Route: in utero (ip to dams prior to mating) Duration: single dose; in utero/lactation exposure. Pups not weaned. Sac at 4 w of age.	-	300 mg/kg-d	-	(Wu <i>et al.</i> , 1999)

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Category	Endpoint	Species (sex)	Test article Dose levels	Dosing parameters	NOAEL dose	LOAEL dose	Notes for NOAEL and/or LOAEL	Reference
Humoral immunity	Circulating IgM response (SRBC antigen; hemolysis)	Monkey (females)	Aroclor 1254 0.2 mg/kg-d	Route: oral (juice) Duration: 5/w; 27-28 mo	0.2 mg/kg-d	-	Small grp size (N=2) prevented definitive conclusions	(Tryphonas <i>et al.</i> , 1986)
Humoral immunity	Circulating antibodies (pseudo-rabies virus antigen; serum neutralization)	Rabbit (not specified)	Aroclor 1221 [20 mg/kg-d; estimated]	Route: oral (gavage) Duration: 1/w; 14 w	-	20 mg/kg-d	-	(Koller and Thigpen, 1973)
Humoral immunity	Circulating antibodies (pseudo-rabies virus antigen; serum neutralization)	Rabbit (not specified)	Aroclor 1242 [20 mg/kg-d; estimated]	Route: oral (gavage) Duration: 1/w; 14 w	-	20 mg/kg-d	-	(Koller and Thigpen, 1973)
Humoral immunity	Circulating antibodies (pseudo-rabies virus antigen; serum neutralization)	Rabbit (not specified)	Aroclor 1254 [20 mg/kg-d; estimated]	Route: oral (gavage) Duration: 1/w; 14 w	-	20 mg/kg-d	-	(Koller and Thigpen, 1973)
Humoral immunity	Circulating antibodies (tetanus antigen; radial immunodiffusion)	Monkey (females)	Aroclor 1254 0.1 or 0.4 mg/kg-d	Route: oral (juice) Duration: 3/w; up to 267 d	-	-	Small grp size (N=1 or 2) prevented definitive conclusion but no difference was observed in TT antigen.	(Truelove <i>et al.</i> , 1982)

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Category	Endpoint	Species (sex)	Test article Dose levels	Dosing parameters	NOAEL dose	LOAEL dose	Notes for NOAEL and/or LOAEL	Reference
Humoral immunity	Circulating antibody response (BSA antigen; ELISA)	Mouse (both)	Aroclor 1254 0.167, 16.7 or 41.7 (dams) mg/kg-d	Route: oral (diet) Duration: 1/d; to 8 w of age	41.7 mg/kg-d	-	-	(Talcott and Koller, 1983)
Humoral immunity	Circulating antibody response (BSA antigen; ELISA)	Rat (male)	Aroclor 1254 [0.03 or 0.3 mg/kg-d; estimated]	Route: oral (gavage) Duration: 2/w; 14 w blood collected on d96	-	0.03 mg/kg-d	-	(Koller <i>et al.</i> , 1983a)
Humoral immunity	Circulating antibody response (EIV antigen; hemagglutination)	Goat (both)	PCB126 Maternal: 0.000049 mg/kg-d	Route: in utero and nursing (GD60 through delivery) Duration: 3/w; assessed at 2 to 8 w of age	0.000049 mg/kg-d	-	-	(Lyche <i>et al.</i> , 2006)
Humoral immunity	Circulating antibody response (paratuberculosis antigen; ELISA)	Goat (both)	PCB126 Maternal: 0.000049 mg/kg-d	Route: in utero and nursing (GD60 through delivery) Duration: 3/w; assessed at 2 to 8 w of age	0.000049 mg/kg-d	-	-	(Lyche <i>et al.</i> , 2006)
Humoral immunity	Circulating antibody response (paratuberculosis antigen; ELISA)	Goat (females)	PCB126 Maternal: 0.000049 mg/kg-d	Route: oral (gavage) Duration: 3/w; GD60 through delivery	0.000049 mg/kg-d	-	-	(Lyche <i>et al.</i> , 2006)
Humoral immunity	Circulating antibody response (paratuberculosis antigen; ELISA)	Goat (both)	PCB153 Maternal: 0.098 mg/kg-d	Route: in utero and nursing Duration: 3/w; assessed at 2 to 8 w of age	0.098 mg/kg-d	-	-	(Lyche <i>et al.</i> , 2006)
Humoral immunity	Circulating antibody response (paratuberculosis antigen; ELISA)	Goat (females)	PCB153 Maternal: 0.098 mg/kg-d	Route: oral (gavage) Duration: 3/w; GD60 through delivery	0.098 mg/kg-d	-	-	(Lyche <i>et al.</i> , 2006)

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Category	Endpoint	Species (sex)	Test article Dose levels	Dosing parameters	NOAEL dose	LOAEL dose	Notes for NOAEL and/or LOAEL	Reference
Humoral immunity	Circulating antibody response (pneumococcal antigens; RIA)	Monkey (females)	Aroclor 1254 0.005 to 0.08 mg/kg-d	Route: oral (gelatin capsule) Duration: 1/d; 55 mo	0.08 mg/kg-d	-	-	(Tryphonas <i>et al.</i> , 1991a)
Humoral immunity	Circulating antibody response (SRBC antigen; hemagglutination)	Mouse (males) C57BL/6J (Ah+)	PCB156 10 or 100 mg/kg-d	Route: ip Duration: single dose; sac 5 d after SRBC injection	10 mg/kg-d	100 mg/kg-d	-	(Silkworth <i>et al.</i> , 1984)
Humoral immunity	Circulating antibody response (SRBC antigen; hemagglutination)	Mouse (males) DBA/2 (Ah-)	PCB156 10 or 100 mg/kg-d	Route: ip Duration: single dose; sac 5 d after SRBC injection	100 mg/kg-d	-	-	(Silkworth <i>et al.</i> , 1984)
Humoral immunity	Circulating antibody response (SRBC antigen; hemagglutination)	Mouse (males) C57BL/6J (Ah+)	PCB47 10 or 100 mg/kg-d	Route: ip Duration: single dose; sac 5 d after SRBC injection	100 mg/kg-d	-	-	(Silkworth <i>et al.</i> , 1984)
Humoral immunity	Circulating antibody response (SRBC antigen; hemagglutination)	Mouse (males) DBA/2 (Ah-)	PCB47 10 or 100 mg/kg-d	Route: ip Duration: single dose; sac 5 d after SRBC injection	100 mg/kg-d	-	-	(Silkworth <i>et al.</i> , 1984)
Humoral immunity	Circulating antibody response (SRBC antigen; hemagglutination)	Mouse (male) C57BL/6J (Ah+)	PCB52 10 or 100 mg/kg-d	Route: ip Duration: 2 doses; sac 5 d after SRBC injection	100 mg/kg-d	-	-	(Silkworth and Grabstein, 1982)
Humoral immunity	Circulating antibody response (SRBC antigen; hemagglutination)	Mouse (male) DBA/2 (Ah-)	PCB52 10 or 100 mg/kg-d	Route: ip Duration: 2 doses; sac 5 d after SRBC injection	100 mg/kg-d	-	-	(Silkworth and Grabstein, 1982)

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Category	Endpoint	Species (sex)	Test article Dose levels	Dosing parameters	NOAEL dose	LOAEL dose	Notes for NOAEL and/or LOAEL	Reference
Humoral immunity	Circulating antibody response (SRBC antigen; hemagglutination)	Mouse (males) CByD2F1	PCB77 10 or 100 mg/kg-d	Route: ip Duration: single dose; sac 4-5 d after SRBC injection	10 mg/kg-d	100 mg/kg-d	-	(Silkworth <i>et al.</i> , 1986)
Humoral immunity	Circulating antibody response (SRBC antigen; hemagglutination)	Mouse (male) BALB/cBy	PCB77 10 or 100 mg/kg-d	Route: ip Duration: 2 doses; sac 5 d after SRBC injection	100 mg/kg-d	-	-	(Silkworth and Grabstein, 1982)
Humoral immunity	Circulating antibody response (SRBC antigen; hemagglutination)	Mouse (male) DBA/2	PCB77 10 or 100 mg/kg-d	Route: ip Duration: 2 doses; sac 5 d after SRBC injection	100 mg/kg-d	-	-	(Silkworth and Grabstein, 1982)
Humoral immunity	Circulating antibody response (SRBC antigen; hemagglutination)	Mouse (males) DBA/2	PCB77 10 or 100 mg/kg-d	Route: ip Duration: single dose; sac 4-5 d after SRBC injection	100 mg/kg-d	-	-	(Silkworth <i>et al.</i> , 1986)
Humoral immunity	Circulating antibody response (SRBC antigen; hemagglutination)	Mouse (males) DBA/2 donor & recipient	PCB77 10 or 100 mg/kg-d	Route: ip Duration: single dose; sac 4-5 d after SRBC injection	100 mg/kg-d	-	-	(Silkworth <i>et al.</i> , 1986)
Humoral immunity	Circulating antibody response (SRBC antigen; hemagglutination)	Mouse (males) BALB/cBy	PCB77 10 or 100 mg/kg-d	Route: ip Duration: single dose; sac 4-5 d after SRBC injection	-	10 mg/kg-d	-	(Silkworth <i>et al.</i> , 1986)
Humoral immunity	Circulating antibody response (SRBC antigen; hemagglutination)	Mouse (males) BALB/cBy donor & recipient	PCB77 10 or 100 mg/kg-d	Route: ip Duration: single dose; sac 4-5 d after SRBC injection	-	10 mg/kg-d	-	(Silkworth <i>et al.</i> , 1986)

Category	Endpoint	Species (sex)	Test article Dose levels	Dosing parameters	NOAEL dose	LOAEL dose	Notes for NOAEL and/or LOAEL	Reference
Humoral immunity	Circulating antibody response (SRBC antigen; hemagglutination)	Mouse (males) DBA/2 donor, BALB/cBy recipient	PCB77 10 or 100 mg/kg-d	Route: ip Duration: single dose; sac 4-5 d after SRBC injection	-	10 mg/kg-d	-	(Silkworth <i>et al.</i> , 1986)
Humoral immunity	Circulating antibody response (SRBC antigen; hemagglutination)	Mouse (males) BALB/cBy donor, DBA/2 recipient	PCB77 10 or 100 mg/kg-d	Route: ip Duration: single dose; sac 4-5 d after SRBC injection	-	10 mg/kg-d	-	(Silkworth <i>et al.</i> , 1986)
Humoral immunity	Circulating gamma globulin (electrophoresis)	Monkey (female)	Aroclor 1248 [0.1 or 0.2 mg/kg-d; estimated]	Route: oral (diet) Duration: 1/d; ~16 mo	0.1 mg/kg-d	0.2 mg/kg-d	-	(Thomas and Hinsdill, 1978)
Humoral immunity	Circulating gamma globulin (electrophoresis)	Rabbit (males)	Aroclor 1254 0.18 to 6.54 mg/kg-d	Route: oral (diet) Duration: 1/d; 8 w	6.54 mg/kg-d	-	-	(Street and Sharma, 1975)
Humoral immunity	Circulating IgG (method unspecified)	Rabbit (not specified)	Aroclor 1221 [20 mg/kg-d; estimated]	Route: oral (gavage) Duration: 1/w; 14 w	20 mg/kg-d	-	-	(Koller and Thigpen, 1973)
Humoral immunity	Circulating IgG (method unspecified)	Rabbit (not specified)	Aroclor 1242 [20 mg/kg-d; estimated]	Route: oral (gavage) Duration: 1/w; 14 w	20 mg/kg-d	-	-	(Koller and Thigpen, 1973)

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Category	Endpoint	Species (sex)	Test article Dose levels	Dosing parameters	NOAEL dose	LOAEL dose	Notes for NOAEL and/or LOAEL	Reference
Humoral immunity	Circulating IgG (method unspecified)	Rabbit (not specified)	Aroclor 1254 [20 mg/kg-d; estimated]	Route: oral (gavage) Duration: 1/w; 14 w	20 mg/kg-d	-	-	(Koller and Thigpen, 1973)
Humoral immunity	Circulating IgG (radial immunodiffusion)	Goat (both)	PCB126 Maternal: 0.000049 mg/kg-d	Route: in utero and nursing (GD60 through delivery) Duration: 3/w; assessed at 2 to 8 w of age	0.000049 mg/kg-d	-	-	(Lyche <i>et al.</i> , 2006)
Humoral immunity	Circulating IgG (radial immunodiffusion)	Goat (females)	PCB126 Maternal: 0.000049 mg/kg-d	Route: oral (gavage) Duration: 3/w; GD60 through delivery	-	0.000049 mg/kg-d	-	(Lyche <i>et al.</i> , 2006)
Humoral immunity	Circulating IgG (radial immunodiffusion)	Goat (females)	PCB153 Maternal: 0.098 mg/kg-d	Route: oral (gavage) Duration: 3/w; GD60 through delivery	0.098 mg/kg-d	-	-	(Lyche <i>et al.</i> , 2006)
Humoral immunity	Circulating IgG (radial immunodiffusion)	Goat (both)	PCB153 Maternal: 0.098 mg/kg-d	Route: oral (gavage) Duration: 3/w; assessed at 2 to 8 w of age	-	0.098 mg/kg-d	-	(Lyche <i>et al.</i> , 2006)
Humoral immunity	Circulating IgG response (A. Pyogenes antigen; ELISA)	Goat (both)	PCB126 Maternal: 0.000049 mg/kg-d	Route: in utero and nursing (GD60 through delivery) Duration: 3/w; assessed at 2 to 8 w of age	0.000049 mg/kg-d	-	-	(Lyche <i>et al.</i> , 2006)
Humoral immunity	Circulating IgG response (A. Pyogenes antigen; ELISA)	Goat (both)	PCB153 Maternal: 0.098 mg/kg-d	Route: oral (gavage) Duration: 3/w; assessed at 2 to 8 w of age	-	0.098 mg/kg-d	-	(Lyche <i>et al.</i> , 2006)

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Category	Endpoint	Species (sex)	Test article Dose levels	Dosing parameters	NOAEL dose	LOAEL dose	Notes for NOAEL and/or LOAEL	Reference
Humoral immunity	Circulating IgG response (diphtheria antigen; hemagglutination)	Goat (both)	PCB126 Maternal: 0.000049 mg/kg-d	Route: in utero and nursing (GD60 through delivery) Duration: 3/w; assessed at 2 to 8 w of age	0.000049 mg/kg-d	-	-	(Lyche <i>et al.</i> , 2006)
Humoral immunity	Circulating IgG response (diphtheria antigen; hemagglutination)	Goat (both)	PCB153 Maternal: 0.098 mg/kg-d	Route: in utero and nursing Duration: 3/w; assessed at 2 to 8 w of age	0.098 mg/kg-d	-	-	(Lyche <i>et al.</i> , 2006)
Humoral immunity	Circulating IgG response (KLH antigen; 1° response; ELISA)	Rat (male)	Aroclor 1254 [4.3 or 43 mg/kg-d; estimated]	Route: oral (diet) Duration: 1/d; 9 w blood collected up to 15d after initial KLH injection	4.3 mg/kg-d	43 mg/kg-d	LOAEL observed at day 8; recovered by day 15	(Koller <i>et al.</i> , 1983b)
Humoral immunity	Circulating IgG response (KLH antigen; 2° response; ELISA)	Rat (male)	Aroclor 1254 [4.3 or 43 mg/kg-d; estimated]	Route: oral (diet) Duration: 1/d; 9 w blood collected up to 21d after initial KLH injection	43 mg/kg-d	-	-	(Koller <i>et al.</i> , 1983b)
Humoral immunity	Circulating IgG response (KLH antigen; 2° response; ELISA)	Rat (males)	Aroclor 1254 [4.3 or 43 mg/kg-d; estimated]	Route: oral (diet) Duration: 1/d; 10 w	-	4.3 mg/kg-d	-	(Exon <i>et al.</i> , 1985)
Humoral immunity	Circulating IgG response (KLH antigen; ELISA)	Mouse (pups (both))	Aroclor 1242 [21 mg/kg-d; estimated]	Route: sc Duration: dams (3/wk); dams (2 w)	21 mg/kg-d	-	-	(Arena <i>et al.</i> , 2003)

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Category	Endpoint	Species (sex)	Test article Dose levels	Dosing parameters	NOAEL dose	LOAEL dose	Notes for NOAEL and/or LOAEL	Reference
Humoral immunity	Circulating IgG response (KLH antigen; ELISA)	Mouse (pups (both))	Aroclor 1254 [21 mg/kg-d; estimated]	Route: sc Duration: dams (3/wk); dams (2 w)	21 mg/kg-d	-	-	(Arena <i>et al.</i> , 2003)
Humoral immunity	Circulating IgG response (KLH antigen; ELISA)	Mouse (both (pooled))	Mix (Aroclor 1242:Aroclor 1254 2:1) [4.7 mg/kg-d; estimated]	Route: oral (diet) Duration: 1/d; to 22 w of age	4.7 mg/kg-d	-	-	(Segre <i>et al.</i> , 2002)
Humoral immunity	Circulating IgG response (M. hemolytica antigen; ELISA)	Goat (both)	PCB126 Maternal: 0.000049 mg/kg-d	Route: in utero and nursing (GD60 through delivery) Duration: 3/w; assessed at 2 to 8 w of age	0.000049 mg/kg-d	-	-	(Lyche <i>et al.</i> , 2006)
Humoral immunity	Circulating IgG response (M. hemolytica antigen; ELISA)	Goat (females)	PCB126 Maternal: 0.000049 mg/kg-d	Route: oral (gavage) Duration: 3/w; GD60 through delivery	0.000049 mg/kg-d	-	-	(Lyche <i>et al.</i> , 2006)
Humoral immunity	Circulating IgG response (M. hemolytica antigen; ELISA)	Goat (females)	PCB153 Maternal: 0.098 mg/kg-d	Route: oral (gavage) Duration: 3/w; GD60 through delivery	0.098 mg/kg-d	-	-	(Lyche <i>et al.</i> , 2006)
Humoral immunity	Circulating IgG response (M. hemolytica antigen; ELISA)	Goat (both)	PCB153 Maternal: 0.098 mg/kg-d	Route: oral (gavage) Duration: 3/w; assessed at 2 to 8 w of age	-	0.098 mg/kg-d	-	(Lyche <i>et al.</i> , 2006)

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Category	Endpoint	Species (sex)	Test article Dose levels	Dosing parameters	NOAEL dose	LOAEL dose	Notes for NOAEL and/or LOAEL	Reference
Humoral immunity	Circulating IgG response (REO-1 antigen; hemagglutination)	Goat (both)	PCB126 Maternal: 0.000049 mg/kg-d	Route: in utero and nursing (GD60 through delivery) Duration: 3/w; assessed at 2 to 8 w of age	-	0.000049 mg/kg-d	-	(Lyche <i>et al.</i> , 2006)
Humoral immunity	Circulating IgG response (REO-1 antigen; hemagglutination)	Goat (both)	PCB153 Maternal: 0.098 mg/kg-d	Route: oral (gavage) Duration: 3/w; assessed at 2 to 8 w of age	-	0.098 mg/kg-d	-	(Lyche <i>et al.</i> , 2006)
Humoral immunity	Circulating IgG response (SRBC antigen; hemagglutination)	Monkey (females)	Aroclor 1254 0.005 to 0.08 mg/kg-d	Route: oral (gelatin capsule) Duration: 1/d; 55 mo	0.02 mg/kg-d	0.04 mg/kg-d	Dose-related trend for ↓IgG SRBC antibody titers; only 0.040 mg/kg statistically different than control	(Tryphonas <i>et al.</i> , 1991a)
Humoral immunity	Circulating IgG response (SRBC antigen; hemagglutination)	Monkey (females)	Aroclor 1254 0.005 to 0.08 mg/kg-d	Route: oral (gelatin capsule) Duration: 1/d; 23 mo	-	0.005 mg/kg-d	-	(Tryphonas <i>et al.</i> , 1989)
Humoral immunity	Circulating IgG response (SRBC antigen; hemagglutination)	Monkey (females)	Aroclor 1254 0.1 or 0.4 mg/kg-d	Route: oral (juice) Duration: 3/w; up to 267 d	-	-	-	(Truelove <i>et al.</i> , 1982)
Humoral immunity	Circulating IgG response (SRBC antigen; hemagglutination)	Monkey (females)	Aroclor 1254 0.1 or 0.4 mg/kg-d	Route: in utero and nursing Duration: 3/w; 139 d	-	-	-	(Truelove <i>et al.</i> , 1982)

Category	Endpoint	Species (sex)	Test article Dose levels	Dosing parameters	NOAEL dose	LOAEL dose	Notes for NOAEL and/or LOAEL	Reference
Humoral immunity	Circulating IgG response (SRBC antigen; hemagglutination)	Monkey (both) Rhesus	Mix (major PCBs in Canadian human milk) 0.0075 mg/kg-d	Route: oral (formula) Duration: 1/d; 20 wks exposure, 66 wks total (w/ follow-up)	-	0.0075 mg/kg-d	Time-dependent ↓serum IgM & IgG anti-SRBC hemagglutination titer. Authors suspected small # of animals contributed to lack of treatment response	(Arnold <i>et al.</i> , 1999)
Humoral immunity	Circulating IgG response (SRBC antigen; hemagglutination)	Monkey (males) Cynomolgus	Mix (major PCBs in Canadian human milk) 0.0075 mg/kg-d	Route: oral (in corn oil) Duration: 1/d; 20 wks exposure, 66 wks total (w/ follow-up)	0.0075 mg/kg-d	-	Time-dependent ↓serum IgM & IgG anti-SRBC hemagglutination titer. Authors suspected small # of animals contributed to lack of treatment response	(Arnold <i>et al.</i> , 1999)
Humoral immunity	Circulating IgG response (SRBC antigen; hemagglutination)	Rabbit (males)	Aroclor 1254 0.18 to 6.54 mg/kg-d	Route: oral (diet) Duration: 1/d; 8 w	-	6.54 mg/kg-d	-	(Street and Sharma, 1975)

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Category	Endpoint	Species (sex)	Test article Dose levels	Dosing parameters	NOAEL dose	LOAEL dose	Notes for NOAEL and/or LOAEL	Reference
Humoral immunity	Circulating IgG response (tetanus antigen; hemagglutination)	Goat (females)	PCB126 Maternal: 0.000049 mg/kg-d	Route: oral (gavage) Duration: 3/w; GD60 through delivery	0.000049 mg/kg-d	-	-	(Lyche <i>et al.</i> , 2006)
Humoral immunity	Circulating IgG response (tetanus antigen; hemagglutination)	Goat (both)	PCB126 Maternal: 0.000049 mg/kg-d	Route: in utero and nursing (GD60 through delivery) Duration: 3/w; assessed at 2 to 8 w of age	-	0.000049 mg/kg-d	-	(Lyche <i>et al.</i> , 2006)
Humoral immunity	Circulating IgG response (tetanus antigen; hemagglutination)	Goat (both)	PCB153 Maternal: 0.098 mg/kg-d	Route: oral (gavage) Duration: 3/w; assessed at 2 to 8 w of age	0.098 mg/kg-d	-	-	(Lyche <i>et al.</i> , 2006)
Humoral immunity	Circulating IgG response (tetanus antigen; hemagglutination)	Goat (females)	PCB153 Maternal: 0.098 mg/kg-d	Route: oral (gavage) Duration: 3/w; GD60 through delivery	-	0.098 mg/kg-d	-	(Lyche <i>et al.</i> , 2006)
Humoral immunity	Circulating IgG response (Tetanus antigen; hemagglutination)	Monkey (female)	Aroclor 1248 [0.1 or 0.2 mg/kg-d; estimated]	Route: oral (diet) Duration: 1/d; ~16 mo	0.2 mg/kg-d	-	-	(Thomas and Hinsdill, 1978)
Humoral immunity	Circulating IgG response EIV-1 antigen; hemagglutination)	Goat (both)	PCB126 Maternal: 0.000049 mg/kg-d	Route: in utero and nursing (GD60 through delivery) Duration: 3/w; assessed at 2 to 8 w of age	0.000049 mg/kg-d	-	-	(Lyche <i>et al.</i> , 2006)
Humoral immunity	Circulating IgG response EIV-1 antigen; hemagglutination)	Goat (females)	PCB126 Maternal: 0.000049 mg/kg-d	Route: oral (gavage) Duration: 3/w; GD60 through delivery	0.000049 mg/kg-d	-	-	(Lyche <i>et al.</i> , 2006)

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Category	Endpoint	Species (sex)	Test article Dose levels	Dosing parameters	NOAEL dose	LOAEL dose	Notes for NOAEL and/or LOAEL	Reference
440	Humoral immunity	Goat (females)	PCB153 Maternal: 0.098 mg/kg-d	Route: oral (gavage) Duration: 3/w; GD60 through delivery	0.098 mg/kg-d	-	-	(Lyche <i>et al.</i> , 2006)
	Humoral immunity	Goat (both)	PCB153 Maternal: 0.098 mg/kg-d	Route: in utero and nursing Duration: 3/w; assessed at 2 to 8 w of age	-	0.098 mg/kg-d	-	(Lyche <i>et al.</i> , 2006)
	Humoral immunity	Goat (both)	PCB153 Maternal: 0.098 mg/kg-d	Route: oral (gavage) Duration: 3/w; assessed at 2 to 8 w of age	-	0.098 mg/kg-d	-	(Lyche <i>et al.</i> , 2006)
	Humoral immunity	Rat (females)	Mix (derived from native herring) Atlantic: 0.3 ngTEQ/kg; Baltic: 1.6 ngTEQ/kg mg/kg-d	Route: oral (diet) Duration: 1/d; 4.5 mo	-	-	-	(Ross <i>et al.</i> , 1996)
	Humoral immunity	Monkey (females)	Aroclor 1254 0.005 to 0.08 mg/kg-d	Route: oral (gelatin capsule) Duration: 1/d; 23 mo	0.08 mg/kg-d	-	-	(Tryphonas <i>et al.</i> , 1989)

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Category	Endpoint	Species (sex)	Test article Dose levels	Dosing parameters	NOAEL dose	LOAEL dose	Notes for NOAEL and/or LOAEL	Reference
Humoral immunity	Circulating IgG, IgM, IgA (immunoplates [ELISA])	Monkey (females)	Aroclor 1254 0.2 mg/kg-d	Route: oral (juice) Duration: 5/w; 12-13 mo	0.2 mg/kg-d	-	Small grp size (N=2) prevented definitive conclusions but no difference in serum IgG and IgA. Trend for ↓IgM with 0.2 mg/kg	(Tryphonas <i>et al.</i> , 1986)
Humoral immunity	Circulating IgG, IgM, IgA (immunoplates [ELISA])	Monkey (females)	Aroclor 1254 0.2 mg/kg-d	Route: oral (juice) Duration: 5/w; 27-28 mo	0.2 mg/kg-d	-	Small grp size (N=2) prevented definitive conclusions but no difference in serum IgG and IgA. Trend for ↓IgM with 0.2 mg/kg	(Tryphonas <i>et al.</i> , 1986)
Humoral immunity	Circulating IgG, IgM, IgA (radial immunodiffusion, RID)	Mouse (males)	Aroclor 1242 [30 mg/kg-d; estimated]	Route: oral (diet) Duration: 1/d; sac 4-7 days following SRBC challenge	30 mg/kg-d	-	-	(Loose <i>et al.</i> , 1977)
Humoral immunity	Circulating IgG, IgM, IgA (radial immunodiffusion, RID)	Mouse (males)	Aroclor 1242 [30 mg/kg-d; estimated]	Route: oral (diet) Duration: 1/d; sac 4-7 days following SRBC injection	-	30 mg/kg-d	-	(Loose <i>et al.</i> , 1977)

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Category	Endpoint	Species (sex)	Test article Dose levels	Dosing parameters	NOAEL dose	LOAEL dose	Notes for NOAEL and/or LOAEL	Reference
Humoral immunity	Circulating IgG, IgM, IgA (radial immunodiffusion, RID)	Mouse (males)	Aroclor 1242 [30 mg/kg-d; estimated]	Route: oral (diet) Duration: 1/d; sac 4-11 days following SRBC injection	-	30 mg/kg-d	-	(Loose <i>et al.</i> , 1977)
Humoral immunity	Circulating IgG1 response (OVA antigen; ELISA)	Mouse (female)	PCB126 0.2 mg/kg-d	Route: oral (gavage) Duration: single dose; sac 10d after treatment	0.2 mg/kg-d	-	-	(Pan <i>et al.</i> , 2004)
Humoral immunity	Circulating IgG1 response (OVA antigen; ELISA)	Mouse (female)	PCB169 2 or 5 mg/kg-d	Route: oral (gavage) Duration: single dose; sac 10d after treatment	5 mg/kg-d	-	-	(Pan <i>et al.</i> , 2004)
Humoral immunity	Circulating IgM (radial immunodiffusion)	Rabbit (males)	Aroclor 1221 [15 mg/kg-d; estimated]	Route: oral (drinking water) Duration: 1/d; 3 mo	-	15 mg/kg-d	-	(Wasserman <i>et al.</i> , 1973)
Humoral immunity	Circulating IgM response (LPS antigen)	Mouse (males) DBA/2	PCB126 0.0012 to 0.12 mg/kg-d	Route: ip Duration: single dose; sac 4 d after immunization	0.0012 mg/kg-d	0.012 mg/kg-d	-	(Harper <i>et al.</i> , 1994)
Humoral immunity	Circulating IgM response (LPS antigen)	Mouse (males) C57BL/6	PCB126 0.00012 to 0.024 mg/kg-d	Route: ip Duration: single dose; sac 4 d after immunization	0.012 mg/kg-d	0.024 mg/kg-d	-	(Harper <i>et al.</i> , 1994)
Humoral immunity	Circulating IgM response (LPS antigen)	Mouse (males)	PCB126 0.006 mg/kg-d	Route: ip Duration: single dose; 6 d post treatment	-	0.006 mg/kg-d	-	(Zhao <i>et al.</i> , 1997)

Category	Endpoint	Species (sex)	Test article Dose levels	Dosing parameters	NOAEL dose	LOAEL dose	Notes for NOAEL and/or LOAEL	Reference
Humoral immunity	Circulating IgM response (LPS antigen)	Mouse (males)	PCB126+PCB153 0.006 mg/kg PCB126 + 18, 36, or 72 mg/kg PCB153 mg/kg-d	Route: ip Duration: single dose; 6 d post treatment	-	-	PCB126-induced ↓ inhibited by co-treatment of PCB153 in a dose-dependent manner	(Zhao <i>et al.</i> , 1997)
Humoral immunity	Circulating IgM response (LPS antigen)	Mouse (males)	PCB153 72 mg/kg-d	Route: ip Duration: single dose; 6 d post treatment	72 mg/kg-d	-	-	(Zhao <i>et al.</i> , 1997)
Humoral immunity	Circulating IgM response (LPS antigen)	Mouse (males) DBA/2	PCB169 0.0024 to 0.096 mg/kg-d	Route: ip Duration: single dose; sac 4 d after immunization	0.024 mg/kg-d	0.048 mg/kg-d	-	(Harper <i>et al.</i> , 1994)
Humoral immunity	Circulating IgM response (LPS antigen)	Mouse (males) C57BL/6	PCB169 0.00024 to 0.048 mg/kg-d	Route: ip Duration: single dose; sac 4 d after immunization	-	0.00024 mg/kg-d	-	(Harper <i>et al.</i> , 1994)
Humoral immunity	Circulating IgM response (OVA antigen; ELISA)	Mouse (female)	PCB126 0.2 mg/kg-d	Route: oral (gavage) Duration: single dose; sac 10d after treatment	-	0.2 mg/kg-d	-	(Pan <i>et al.</i> , 2004)
Humoral immunity	Circulating IgM response (SRBC antigen; ELISA)	Mouse (females)	PCB126 0.01 to 1 mg/kg-d	Route: oral (gavage) Duration: single dose; sac 4d (PFC) or 5d (serum IgM-SRBC) after immunization	-	0.01 mg/kg-d	-	(Johnson <i>et al.</i> , 2000)

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Category	Endpoint	Species (sex)	Test article Dose levels	Dosing parameters	NOAEL dose	LOAEL dose	Notes for NOAEL and/or LOAEL	Reference
Humoral immunity	Circulating IgM response (SRBC antigen; hemolysis)	Monkey (female)	Aroclor 1248 [0.1 or 0.2 mg/kg-d; estimated]	Route: oral (diet) Duration: 1/d; ~16 mo	0.1 mg/kg-d	0.2 mg/kg-d	-	(Thomas and Hinsdill, 1978)
Humoral immunity	Circulating IgM response (SRBC antigen; hemolysis)	Monkey (females)	Aroclor 1254 0.2 mg/kg-d	Route: oral (juice) Duration: 5/w; 12-13 mo	0.2 mg/kg-d	-	Small grp size (N=2) prevented definitive conclusions	(Tryphonas <i>et al.</i> , 1986)
Humoral immunity	Circulating IgM response (SRBC antigen; hemolysis)	Monkey (females)	Aroclor 1254 0.005 to 0.08 mg/kg-d	Route: oral (gelatin capsule) Duration: 1/d; 55 mo	-	0.005 mg/kg-d	-	(Tryphonas <i>et al.</i> , 1991a)
Humoral immunity	Circulating IgM response (SRBC antigen; hemolysis)	Monkey (females)	Aroclor 1254 0.005 to 0.08 mg/kg-d	Route: oral (gelatin capsule) Duration: 1/d; 23 mo	-	0.005 mg/kg-d	All groups except 0.020 mg/kg statistically different than control	(Tryphonas <i>et al.</i> , 1989)
Humoral immunity	Lymphocyte blastogenesis, B cell mitogen(s): LPS	Goat (both)	PCB126 Maternal: 0.000049 mg/kg-d	Route: in utero and nursing (GD60 through lactation) Duration: 3/w; sac at 9 mo of age	0.000049 mg/kg-d	-	-	(Lyche <i>et al.</i> , 2004)
Humoral immunity	Lymphocyte blastogenesis, B cell mitogen(s): LPS	Goat (both)	PCB153 Maternal: 0.098 mg/kg-d	Route: in utero and nursing (GD60 through lactation) Duration: 3/w; sac at 9 mo of age	0.098 mg/kg-d	-	-	(Lyche <i>et al.</i> , 2004)
Humoral immunity	Lymphocyte blastogenesis, B cell mitogen(s): LPS	Mouse (male)	Aroclor 1016 [30 mg/kg-d; estimated]	Route: oral (diet) Duration: 1/d; 40 w	30 mg/kg-d	-	-	(Silkworth and Loose, 1981)

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Category	Endpoint	Species (sex)	Test article Dose levels	Dosing parameters	NOAEL dose	LOAEL dose	Notes for NOAEL and/or LOAEL	Reference
Humoral immunity	Lymphocyte blastogenesis, B cell mitogen(s): LPS	Mouse (males)	Aroclor 1016 [30 mg/kg-d; estimated]	Route: oral (diet) Duration: 1/d; 41w	30 mg/kg-d	-	-	(Silkworth and Loose, 1979a)
Humoral immunity	Lymphocyte blastogenesis, B cell mitogen(s): LPS	Mouse (male)	Aroclor 1254 63 to 550 mg/kg-d	Route: ip Duration: single dose; injection 1 w before sac	550 mg/kg-d	-	-	(Wierda <i>et al.</i> , 1981)
Humoral immunity	Lymphocyte blastogenesis, B cell mitogen(s): LPS	Mouse (both)	Fenclo 42 500 or 1,000 mg/kg-d	Route: ip Duration: 1/d; single dose	-	500 mg/kg-d	Recovered to baseline 5 to 8 d after exposure	(Franco <i>et al.</i> , 1989)
Humoral immunity	Lymphocyte blastogenesis, B cell mitogen(s): LPS	Mouse (both)	Fenclo 42 500 or 1,000 mg/kg-d	Route: ip Duration: 1/d; 15d	-	500 mg/kg-d	Recovered to baseline 5 to 8 d after exposure	(Franco <i>et al.</i> , 1989)
Humoral immunity	Lymphocyte blastogenesis, B cell mitogen(s): LPS	Mouse (males)	PCB153 0.00009 to 1.4 mg/kg-d	Route: oral (diet) Duration: 1/d; 2w prior to mating through PND21; offspring followed for 1 yr	-	-	Analysis limited to trend analysis. No dose/response observed.	(van Esterik <i>et al.</i> , 2015)
Humoral immunity	Lymphocyte blastogenesis, B cell mitogen(s): LPS	Mouse (females)	PCB153 0.00009 to 1.4 mg/kg-d	Route: oral (diet) Duration: 1/d; 2w prior to mating through PND21; offspring followed for 1 yr	-	-	Analysis limited to trend analysis. No dose/response observed.	(van Esterik <i>et al.</i> , 2015)

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Category	Endpoint	Species (sex)	Test article Dose levels	Dosing parameters	NOAEL dose	LOAEL dose	Notes for NOAEL and/or LOAEL	Reference
Humoral immunity	Lymphocyte blastogenesis, B cell mitogen(s): STM	Mouse (females)	PCB118 82 to 260 mg/kg-d	Route: oral (gavage) Duration: single dose; sac 2 d after treatment	260 mg/kg-d	-	-	(Dahlman <i>et al.</i> , 1994)
Humoral immunity	Lymphocyte blastogenesis, B cell mitogen(s): STM	Mouse (females)	PCB77 7.3 to 29 mg/kg-d	Route: oral (gavage) Duration: single dose; sac 2 d after treatment	29 mg/kg-d	-	-	(Dahlman <i>et al.</i> , 1994)
Humoral immunity	Lymphocyte blastogenesis, B cell mitogen(s): STM	Rat (males)	Aroclor 1254 0.1 to 25 mg/kg-d	Route: oral (gavage) Duration: 1/d; 15w	25 mg/kg-d	-	-	(Smialowicz <i>et al.</i> , 1989)
Humoral immunity	Lymphocyte blastogenesis, B,T cell mitogen(s): PWM	Goat (both)	PCB126 Maternal: 0.000049 mg/kg-d	Route: in utero and nursing (GD60 through lactation) Duration: 3/w; sac at 9 mo of age	0.000049 mg/kg-d	-	-	(Lyche <i>et al.</i> , 2004)
Humoral immunity	Lymphocyte blastogenesis, B,T cell mitogen(s): PWM	Goat (both)	PCB153 Maternal: 0.098 mg/kg-d	Route: in utero and nursing (GD60 through lactation) Duration: 3/w; sac at 9 mo of age	0.098 mg/kg-d	-	-	(Lyche <i>et al.</i> , 2004)
Humoral immunity	Lymphocyte blastogenesis, B,T cell mitogen(s): PWM	Monkey (females)	Aroclor 1254 0.005 to 0.08 mg/kg-d	Route: oral (gelatin capsule) Duration: 1/d; 55 mo	0.08 mg/kg-d	-	-	(Tryphonas <i>et al.</i> , 1991a)
Humoral immunity	Lymphocyte blastogenesis, B,T cell mitogen(s): PWM	Monkey (both)	Mix (major PCBs in Canadian human milk) 0.0075 mg/kg-d	Route: oral (in corn oil) Duration: 1/d; 20 wks exposure, 66 wks total (w/ follow-up)	0.0075 mg/kg-d	-	-	(Arnold <i>et al.</i> , 1999)

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Category	Endpoint	Species (sex)	Test article Dose levels	Dosing parameters	NOAEL dose	LOAEL dose	Notes for NOAEL and/or LOAEL	Reference
Humoral immunity	Lymphocyte blastogenesis, B,T cell mitogen(s): PWM	Mouse (both)	Fenclor 42 500 or 1,000 mg/kg-d	Route: ip Duration: 1/d; single dose	500 mg/kg-d	1000 mg/kg-d	-	(Franco <i>et al.</i> , 1989)
Humoral immunity	Lymphocyte blastogenesis, B,T cell mitogen(s): PWM	Mouse (both)	Fenclor 42 500 or 1,000 mg/kg-d	Route: ip Duration: 1/d; 15d	-	500 mg/kg-d	-	(Franco <i>et al.</i> , 1989)
Humoral immunity	Lymphocyte blastogenesis, B,T cell mitogen(s): PWM	Rat (males)	Aroclor 1254 [22 mg/kg-d; estimated]	Route: oral (diet) Duration: 1/d; 7 d	22 mg/kg-d	-	-	(Bonnyns and Bastomsky, 1976)
Humoral immunity	Lymphocyte blastogenesis, B,T cell mitogen(s): PWM	Rat (males)	Aroclor 1254 0.1 to 25 mg/kg-d	Route: oral (gavage) Duration: 1/d; 15w	25 mg/kg-d	-	-	(Smialowicz <i>et al.</i> , 1989)
Humoral immunity	Lymphocyte blastogenesis, B,T cell mitogen(s): PWM	Rat (females)	Mix (derived from native herring oil) Pups cumulative intake at 11d (2.03, 19.9 ng TEQ/kg Atlantic, Baltic oils); at 25 d (1.83, 17.3 ng TEQ/kg Atlantic, Baltic oils) mg/kg-d	Route: oral (gavage) Duration: 1/d; GD6 to pup weaning (41 exposure days)	-	-	-	(Ross <i>et al.</i> , 1997)

Category	Endpoint	Species (sex)	Test article Dose levels	Dosing parameters	NOAEL dose	LOAEL dose	Notes for NOAEL and/or LOAEL	Reference
Humoral immunity	Lymphocyte blastogenesis, B,T cell mitogen(s): PWM	Rat (females)	Mix (derived from native herring) Atlantic: 0.3 ngTEQ/kg; Baltic: 1.6 ngTEQ/kg mg/kg-d	Route: oral (diet) Duration: 1/d; 4.5 mo	-	-	-	(Ross <i>et al.</i> , 1996)
Humoral immunity	PFC response (Polyclonal; LPS antigen)	Mouse (females)	Aroclor 1242 50 to 1000 mg/kg-d	Route: ip Duration: single dose; 6 d post treatment	100 mg/kg-d	500 mg/kg-d	-	(Harper <i>et al.</i> , 1995)
Humoral immunity	PFC response (Polyclonal; LPS antigen)	Mouse (females)	Aroclor 1248 50 to 1000 mg/kg-d	Route: ip Duration: single dose; 6 d post treatment	-	50 mg/kg-d	-	(Harper <i>et al.</i> , 1995)
Humoral immunity	PFC response (Polyclonal; LPS antigen)	Mouse (females)	Aroclor 1254 50 to 1000 mg/kg-d	Route: ip Duration: single dose; 6 d post treatment	50 mg/kg-d	100 mg/kg-d	-	(Harper <i>et al.</i> , 1995)
Humoral immunity	PFC response (Polyclonal; LPS antigen)	Mouse (females)	Aroclor 1260 50 to 1000 mg/kg-d	Route: ip Duration: single dose; 6 d post treatment	-	50 mg/kg-d	-	(Harper <i>et al.</i> , 1995)
Humoral immunity	PFC response (Polyclonal; LPS antigen)	Mouse (females)	PCB105 25 to 150 mg/kg-d	Route: ip Duration: single dose; 6 d post treatment	25 mg/kg-d	75 mg/kg-d	-	(Harper <i>et al.</i> , 1995)

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Category	Endpoint	Species (sex)	Test article Dose levels	Dosing parameters	NOAEL dose	LOAEL dose	Notes for NOAEL and/or LOAEL	Reference
449	Humoral immunity	Mouse (females)	PCB118 25 to 150 mg/kg-d	Route: ip Duration: single dose; 6 d post treatment	75 mg/kg-d	150 mg/kg-d	-	(Harper <i>et al.</i> , 1995)
	Humoral immunity	Mouse (males)	PCB126 0.00012 to 0.024 mg/kg-d	Route: ip Duration: single dose; sac 4 d after immunization	0.00012 mg/kg-d	0.0012 mg/kg-d	-	(Harper <i>et al.</i> , 1993a)
	Humoral immunity	Mouse (females)	PCB126 0.00012 to 0.012 mg/kg-d	Route: ip Duration: single dose; 6 d post treatment	0.0012 mg/kg-d	0.0024 mg/kg-d	-	(Harper <i>et al.</i> , 1995)
	Humoral immunity	Mouse (males)	PCB126 0.00012 to 0.012 mg/kg-d	Route: ip Duration: single dose; sac 4 d after immunization	0.012 mg/kg-d	0.024 mg/kg-d	-	(Harper <i>et al.</i> , 1993a)
	Humoral immunity	Mouse (males)	PCB126 0.006 mg/kg-d	Route: ip Duration: single dose; 6 d post treatment	-	0.006 mg/kg-d	-	(Zhao <i>et al.</i> , 1997)
	Humoral immunity	Mouse (males)	PCB126+PCB153 0.006 mg/kg PCB126 + 18, 36, or 72 mg/kg PCB153 mg/kg-d	Route: ip Duration: single dose; 6 d post treatment	-	-	-	(Zhao <i>et al.</i> , 1997)
	Humoral immunity	Mouse (females)	PCB153 9 to 72 mg/kg-d	Route: ip Duration: single dose; 6 d post treatment	72 mg/kg-d	-	-	(Harper <i>et al.</i> , 1995)

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Category	Endpoint	Species (sex)	Test article Dose levels	Dosing parameters	NOAEL dose	LOAEL dose	Notes for NOAEL and/or LOAEL	Reference
Humoral immunity	PFC response (Polyclonal; LPS antigen)	Mouse (males)	PCB153 72 mg/kg-d	Route: ip Duration: single dose; 6 d post treatment	72 mg/kg-d	-	-	(Zhao <i>et al.</i> , 1997)
Humoral immunity	PFC response (Polyclonal; LPS antigen)	Mouse (females)	PCB156 25 to 150 mg/kg-d	Route: ip Duration: single dose; 6 d post treatment	25 mg/kg-d	75 mg/kg-d	-	(Harper <i>et al.</i> , 1995)
Humoral immunity	PFC response (Polyclonal; LPS antigen)	Mouse (males)	PCB169 0.00024 to 0.048 mg/kg-d	Route: ip Duration: single dose; sac 4 d after immunization	0.00024 mg/kg-d	0.0024 mg/kg-d	-	(Harper <i>et al.</i> , 1993a)
Humoral immunity	PFC response (Polyclonal; LPS antigen)	Mouse (females)	PCB169 0.00024 to 0.024 mg/kg-d	Route: ip Duration: single dose; 6 d post treatment	0.00024 mg/kg-d	0.0024 mg/kg-d	-	(Harper <i>et al.</i> , 1995)
Humoral immunity	PFC response (Polyclonal; LPS antigen)	Mouse (males)	PCB169 0.00024 to 0.069 mg/kg-d	Route: ip Duration: single dose; sac 4 d after immunization	0.024 mg/kg-d	0.048 mg/kg-d	-	(Harper <i>et al.</i> , 1993a)
Humoral immunity	PFC response (Polyclonal; LPS antigen)	Mouse (females)	PCB170 25 to 200 mg/kg-d	Route: ip Duration: single dose; 6 d post treatment	50 mg/kg-d	100 mg/kg-d	-	(Harper <i>et al.</i> , 1995)
Humoral immunity	PFC response (Polyclonal; LPS antigen)	Mouse (females)	PCB180 50 to 200 mg/kg-d	Route: ip Duration: single dose; 6 d post treatment	50 mg/kg-d	100 mg/kg-d	-	(Harper <i>et al.</i> , 1995)

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Category	Endpoint	Species (sex)	Test article Dose levels	Dosing parameters	NOAEL dose	LOAEL dose	Notes for NOAEL and/or LOAEL	Reference
Humoral immunity	PFC response (Polyclonal; LPS antigen)	Mouse (females)	PCB189 25 to 200 mg/kg-d	Route: ip Duration: single dose; 6 d post treatment	25 mg/kg-d	50 mg/kg-d	-	(Harper <i>et al.</i> , 1995)
Humoral immunity	PFC response (Polyclonal; LPS antigen)	Mouse (females)	PCB77 0.024 to 1.2 mg/kg-d	Route: ip Duration: single dose; 6 d post treatment	0.024 mg/kg-d	.12 mg/kg-d	-	(Harper <i>et al.</i> , 1995)
Humoral immunity	PFC response (T-dependent; SRBC antigen)	Mouse (male)	Aroclor 1016 50 to 900 mg/kg-d	Route: ip Duration: single dose; sac 4 d after SRBC	-	-	-	(Davis and Safe, 1989)
Humoral immunity	PFC response (T-dependent; SRBC antigen)	Mouse (male)	Aroclor 1232 50 to 900 mg/kg-d	Route: ip Duration: single dose; sac 4 d after SRBC	-	-	-	(Davis and Safe, 1989)
Humoral immunity	PFC response (T-dependent; SRBC antigen)	Mouse (males)	Aroclor 1242 [30 mg/kg-d; estimated]	Route: oral (diet) Duration: 1/d; sac 4-7 days following SRBC injection	-	30 mg/kg-d	-	(Loose <i>et al.</i> , 1977) (Loose <i>et al.</i> , 1978a)
Humoral immunity	PFC response (T-dependent; SRBC antigen)	Mouse (males)	Aroclor 1242 [30 mg/kg-d; estimated]	Route: oral (diet) Duration: 1/d; sac 4-7 days following SRBC injection	-	30 mg/kg-d	-	(Loose <i>et al.</i> , 1977) (Loose <i>et al.</i> , 1978a)
Humoral immunity	PFC response (T-dependent; SRBC antigen)	Mouse (males)	Aroclor 1242 [30 mg/kg-d; estimated]	Route: oral (diet) Duration: 1/d; sac 4-11 days following SRBC injection	-	30 mg/kg-d	-	(Loose <i>et al.</i> , 1977) (Loose <i>et al.</i> , 1978a)

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Category	Endpoint	Species (sex)	Test article Dose levels	Dosing parameters	NOAEL dose	LOAEL dose	Notes for NOAEL and/or LOAEL	Reference
Humoral immunity	PFC response (T-dependent; SRBC antigen)	Mouse (male)	Aroclor 1242 50 to 900 mg/kg-d	Route: ip Duration: single dose; sac 4 d after SRBC	-	-	-	(Davis and Safe, 1989)
Humoral immunity	PFC response (T-dependent; SRBC antigen)	Mouse (male)	Aroclor 1248 50 to 900 mg/kg-d	Route: ip Duration: single dose; sac 4 d after SRBC	-	-	-	(Davis and Safe, 1989)
Humoral immunity	PFC response (T-dependent; SRBC antigen)	Mouse (male)	Aroclor 1254 1.6 to 49 mg/kg-d	Route: ip Duration: single dose; sac 10 d after SRBC	49 mg/kg-d	-	-	(Bannister <i>et al.</i> , 1987)
Humoral immunity	PFC response (T-dependent; SRBC antigen)	Mouse (not specified)	Aroclor 1254 125 to 750 mg/kg-d	Route: ip Duration: single dose; sac 4-5 d after immunization	125 mg/kg-d	250 mg/kg-d	-	(Lubet <i>et al.</i> , 1986)
Humoral immunity	PFC response (T-dependent; SRBC antigen)	Mouse (not specified)	Aroclor 1254 125 to 750 mg/kg-d	Route: ip Duration: single dose; sac 4-5 d after immunization	125 mg/kg-d	250 mg/kg-d	-	(Lubet <i>et al.</i> , 1986)
Humoral immunity	PFC response (T-dependent; SRBC antigen)	Mouse (not specified)	Aroclor 1254 500 or 750 mg/kg-d	Route: ip Duration: single dose; sac 4-5 d after immunization	750 mg/kg-d	-	-	(Lubet <i>et al.</i> , 1986)
Humoral immunity	PFC response (T-dependent; SRBC antigen)	Mouse (male)	Aroclor 1254 63 to 550 mg/kg-d	Route: ip Duration: single dose; injection 1 w before sac	-	63 mg/kg-d	-	(Wierda <i>et al.</i> , 1981)

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Category	Endpoint	Species (sex)	Test article Dose levels	Dosing parameters	NOAEL dose	LOAEL dose	Notes for NOAEL and/or LOAEL	Reference
Humoral immunity	PFC response (T-dependent; SRBC antigen)	Mouse (not specified)	Aroclor 1254 250 to 1500 mg/kg-d	Route: ip Duration: single dose; sac 7, 42, or 102 d post-PCB treatment	-	250 mg/kg-d	-	(Lubet <i>et al.</i> , 1986)
Humoral immunity	PFC response (T-dependent; SRBC antigen)	Mouse (not specified)	Aroclor 1254 500 mg/kg-d	Route: ip Duration: single dose; sac 4-5 d after immunization	-	500 mg/kg-d	-	(Lubet <i>et al.</i> , 1986)
Humoral immunity	PFC response (T-dependent; SRBC antigen)	Mouse (male)	Aroclor 1254 50 to 900 mg/kg-d	Route: ip Duration: single dose; sac 4 d after SRBC	-	-	-	(Davis and Safe, 1989)
Humoral immunity	PFC response (T-dependent; SRBC antigen)	Mouse (male)	Aroclor 1260 50 to 900 mg/kg-d	Route: ip Duration: single dose; sac 4 d after SRBC	-	-	-	(Davis and Safe, 1989)
Humoral immunity	PFC response (T-dependent; SRBC antigen)	Mouse (male)	Mix (major PCBs in human milk) 5 to 50 mg/kg-d	Route: ip Duration: single dose; sac 4 d after SRBC	50 mg/kg-d	-	-	(Davis and Safe, 1989)
Humoral immunity	PFC response (T-dependent; SRBC antigen)	Mouse (male)	PCB108 15 to 65 mg/kg-d	Route: ip Duration: single dose; sac 4 d after SRBC	33 mg/kg-d	65 mg/kg-d	-	(Davis and Safe, 1990)
Humoral immunity	PFC response (T-dependent; SRBC antigen)	Mouse (females)	PCB118 15 to 480 mg/kg-d	Route: oral (gavage) Duration: single dose; sac 4d (PFC) or 5d (serum IgM-SRBC) after immunization	60 mg/kg-d	120 mg/kg-d	-	(Johnson <i>et al.</i> , 2000)

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Category	Endpoint	Species (sex)	Test article Dose levels	Dosing parameters	NOAEL dose	LOAEL dose	Notes for NOAEL and/or LOAEL	Reference
Humoral immunity	PFC response (T-dependent; SRBC antigen)	Mouse (females)	PCB118 82 to 260 mg/kg-d	Route: oral (gavage) Duration: single dose; sac 4 d after SRBC	82 mg/kg-d	260 mg/kg-d	-	(Dahlman <i>et al.</i> , 1994)
Humoral immunity	PFC response (T-dependent; SRBC antigen)	Mouse (females)	PCB118 15 to 480 mg/kg-d	Route: oral (gavage) Duration: single dose; sac 4d (PFC) or 5d (serum IgM-SRBC) after immunization	240 mg/kg-d	480 mg/kg-d	-	(Johnson <i>et al.</i> , 2000)
Humoral immunity	PFC response (T-dependent; SRBC antigen)	Mouse (male)	PCB126 0.00012 to 0.12 mg/kg-d	Route: ip Duration: single dose; sac 5 d after SRBC	-	0.0012 mg/kg-d	-	(Mayura <i>et al.</i> , 1993)
Humoral immunity	PFC response (T-dependent; SRBC antigen)	Mouse (females)	PCB126 0.01 to 1 mg/kg-d	Route: oral (gavage) Duration: single dose; sac 4d (PFC) or 5d (serum IgM-SRBC) after immunization	-	0.01 mg/kg-d	-	(Johnson <i>et al.</i> , 2000)
Humoral immunity	PFC response (T-dependent; SRBC antigen)	Mouse (female)	PCB153 3.58 or 358 mg/kg-d	Route: oral (gavage) Duration: single dose; 7 d following treatment, SRBC injected	35.8 mg/kg-d	358 mg/kg-d	-	(Smialowicz <i>et al.</i> , 1997)
Humoral immunity	PFC response (T-dependent; SRBC antigen)	Mouse (not specified)	PCB153 36 to 360 mg/kg-d	Route: ip Duration: single dose; sac 10 d after chemical treatment	360 mg/kg-d	-	-	(Biegel <i>et al.</i> , 1989)
Humoral immunity	PFC response (T-dependent; SRBC antigen)	Mouse (male)	PCB154 36 to 360 mg/kg-d	Route: ip Duration: single dose; sac 4 d after SRBC	360 mg/kg-d	-	-	(Davis and Safe, 1990)

Category	Endpoint	Species (sex)	Test article Dose levels	Dosing parameters	NOAEL dose	LOAEL dose	Notes for NOAEL and/or LOAEL	Reference
Humoral immunity	PFC response (T-dependent; SRBC antigen)	Mouse (male)	PCB155	Route: ip	360 mg/kg-d	-	-	(Davis and Safe, 1990)
			180 to 360 mg/kg-d	Duration: single dose; sac 4 d after SRBC				
Humoral immunity	PFC response (T-dependent; SRBC antigen)	Mouse (male)	PCB156	Route: ip	0.36 mg/kg-d	1.8 mg/kg-d	-	(Davis and Safe, 1990)
			0.36 to 36 mg/kg-d	Duration: single dose; sac 4 d after SRBC				
Humoral immunity	PFC response (T-dependent; SRBC antigen)	Mouse (males)	PCB156	Route: ip	10 mg/kg-d	100 mg/kg-d	-	(Silkworth <i>et al.</i> , 1984)
			10 or 100 mg/kg-d	Duration: single dose; sac 5 d after SRBC injection				
Humoral immunity	PFC response (T-dependent; SRBC antigen)	Mouse (males)	PCB156	Route: ip	100 mg/kg-d	-	-	(Silkworth <i>et al.</i> , 1984)
			10 or 100 mg/kg-d	Duration: single dose; sac 5 d after SRBC injection				
Humoral immunity	PFC response (T-dependent; SRBC antigen)	Mouse (males)	PCB156	Route: ip	-	250 mg/kg-d	-	(Silkworth <i>et al.</i> , 1984)
			250 or 380 mg/kg-d	Duration: single dose; sac 5 d after SRBC injection				
Humoral immunity	PFC response (T-dependent; SRBC antigen)	Mouse (male)	PCB159	Route: ip	36 mg/kg-d	72 mg/kg-d	-	(Davis and Safe, 1990)
			7.2 to 72 mg/kg-d	Duration: single dose; sac 4 d after SRBC				
Humoral immunity	PFC response (T-dependent; SRBC antigen)	Mouse (male)	PCB168	Route: ip	18 mg/kg-d	36 mg/kg-d	-	(Davis and Safe, 1990)
			7.2 to 72 mg/kg-d	Duration: single dose; sac 4 d after SRBC				

Category	Endpoint	Species (sex)	Test article Dose levels	Dosing parameters	NOAEL dose	LOAEL dose	Notes for NOAEL and/or LOAEL		Reference
456	Humoral immunity	PFC response (T-dependent; SRBC antigen)	Mouse (male)	PCB169	Route: ip	0.00024 mg/kg-d	0.0024 mg/kg-d	-	(Mayura <i>et al.</i> , 1993)
				0.00024 to 0.24 mg/kg-d	Duration: single dose; sac 5 d after SRBC				
	Humoral immunity	PFC response (T-dependent; SRBC antigen)	Mouse (males)	PCB206	Route: ip	4.6 mg/kg-d	12 mg/kg-d	-	(Harper <i>et al.</i> , 1993b)
				4.6 to 46 mg/kg-d	Duration: single dose; sac 4 d after immunization				
	Humoral immunity	PFC response (T-dependent; SRBC antigen)	Mouse (males)	PCB206	Route: ip	12 mg/kg-d	46 mg/kg-d	-	(Harper <i>et al.</i> , 1993b)
				12 to 190 mg/kg-d	Duration: single dose; sac 4 d after immunization				
	Humoral immunity	PFC response (T-dependent; SRBC antigen)	Mouse (males)	PCB207	Route: ip	46 mg/kg-d	190 mg/kg-d	-	(Harper <i>et al.</i> , 1993b)
				12 to 190 mg/kg-d	Duration: single dose; sac 4 d after immunization				
	Humoral immunity	PFC response (T-dependent; SRBC antigen)	Mouse (males)	PCB207	Route: ip	-	4.6 mg/kg-d	-	(Harper <i>et al.</i> , 1993b)
				4.6 to 46 mg/kg-d	Duration: single dose; sac 4 d after immunization				
	Humoral immunity	PFC response (T-dependent; SRBC antigen)	Mouse (males)	PCB208	Route: ip	12 mg/kg-d	46 mg/kg-d	-	(Harper <i>et al.</i> , 1993b)
				12 to 190 mg/kg-d	Duration: single dose; sac 4 d after immunization				
	Humoral immunity	PFC response (T-dependent; SRBC antigen)	Mouse (males)	PCB208	Route: ip	-	4.6 mg/kg-d	-	(Harper <i>et al.</i> , 1993b)
				4.6 to 46 mg/kg-d	Duration: single dose; sac 4 d after immunization				

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Category	Endpoint	Species (sex)	Test article Dose levels	Dosing parameters	NOAEL dose	LOAEL dose	Notes for NOAEL and/or LOAEL	Reference
Humoral immunity	PFC response (T-dependent; SRBC antigen)	Mouse (males)	PCB209 5 to 50 mg/kg-d	Route: ip Duration: single dose; sac 4 d after immunization	12 mg/kg-d	50 mg/kg-d	-	(Harper <i>et al.</i> , 1993b)
Humoral immunity	PFC response (T-dependent; SRBC antigen)	Mouse (males)	PCB209 12 to 200 mg/kg-d	Route: ip Duration: single dose; sac 4 d after immunization	-	12 mg/kg-d	-	(Harper <i>et al.</i> , 1993b)
Humoral immunity	PFC response (T-dependent; SRBC antigen)	Mouse (males)	PCB47 10 or 100 mg/kg-d	Route: ip Duration: single dose; sac 5 d after SRBC injection	100 mg/kg-d	-	-	(Silkworth <i>et al.</i> , 1984)
Humoral immunity	PFC response (T-dependent; SRBC antigen)	Mouse (males)	PCB47 10 or 100 mg/kg-d	Route: ip Duration: single dose; sac 5 d after SRBC injection	100 mg/kg-d	-	-	(Silkworth <i>et al.</i> , 1984)
Humoral immunity	PFC response (T-dependent; SRBC antigen)	Mouse (male)	PCB52 10 or 100 mg/kg-d	Route: ip Duration: 2 doses; sac 5 d after SRBC injection	100 mg/kg-d	-	-	(Silkworth and Grabstein, 1982)
Humoral immunity	PFC response (T-dependent; SRBC antigen)	Mouse (male)	PCB52 10 or 100 mg/kg-d	Route: ip Duration: 2 doses; sac 5 d after SRBC injection	100 mg/kg-d	-	-	(Silkworth and Grabstein, 1982)
Humoral immunity	PFC response (T-dependent; SRBC antigen)	Mouse (male)	PCB77 0.0012 to 1.2 mg/kg-d	Route: ip Duration: single dose; sac 5 d after SRBC	0.0012 mg/kg-d	0.012 mg/kg-d	-	(Mayura <i>et al.</i> , 1993)

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Category	Endpoint	Species (sex)	Test article Dose levels	Dosing parameters	NOAEL dose	LOAEL dose	Notes for NOAEL and/or LOAEL	Reference
Humoral immunity	PFC response (T-dependent; SRBC antigen)	Mouse (male)	PCB77 10 or 100 mg/kg-d	Route: ip Duration: 2 doses; sac 5 d after SRBC injection	10 mg/kg-d	100 mg/kg-d	-	(Silkworth and Grabstein, 1982)
Humoral immunity	PFC response (T-dependent; SRBC antigen)	Mouse (males) DBA/2 donor & recipient	PCB77 10 or 100 mg/kg-d	Route: ip Duration: single dose; sac 4-5 d after SRBC injection	10 mg/kg-d	100 mg/kg-d	-	(Silkworth <i>et al.</i> , 1986)
Humoral immunity	PFC response (T-dependent; SRBC antigen)	Mouse (male)	PCB77 10 or 100 mg/kg-d	Route: ip Duration: 2 doses; sac 5 d after SRBC injection	100 mg/kg-d	-	-	(Silkworth and Grabstein, 1982)
Humoral immunity	PFC response (T-dependent; SRBC antigen)	Mouse (males) DBA/2	PCB77 10 or 100 mg/kg-d	Route: ip Duration: single dose; sac 4-5 d after SRBC injection	100 mg/kg-d	-	-	(Silkworth <i>et al.</i> , 1986)
Humoral immunity	PFC response (T-dependent; SRBC antigen)	Mouse (males) DBA/2 donor; BALB/cBy recipient	PCB77 10 or 100 mg/kg-d	Route: ip Duration: single dose; sac 4-5 d after SRBC injection	100 mg/kg-d	-	-	(Silkworth <i>et al.</i> , 1986)
Humoral immunity	PFC response (T-dependent; SRBC antigen)	Mouse (females)	PCB77 7.3 or 29 mg/kg-d	Route: oral (gavage) Duration: single dose; sac 4 d after SRBC	-	7.3 mg/kg-d	-	(Dahlman <i>et al.</i> , 1994)
Humoral immunity	PFC response (T-dependent; SRBC antigen)	Mouse (males) BALB/cBy	PCB77 10 or 100 mg/kg-d	Route: ip Duration: single dose; sac 4-5 d after SRBC injection	-	10 mg/kg-d	-	(Silkworth <i>et al.</i> , 1986)

Category	Endpoint	Species (sex)	Test article Dose levels	Dosing parameters	NOAEL dose	LOAEL dose	Notes for NOAEL and/or LOAEL	Reference
Humoral immunity	PFC response (T-dependent; SRBC antigen)	Mouse (males) CByD2F1	PCB77 10 or 100 mg/kg-d	Route: ip Duration: single dose; sac 4-5 d after SRBC injection	-	10 mg/kg-d	-	(Silkworth <i>et al.</i> , 1986)
Humoral immunity	PFC response (T-dependent; SRBC antigen)	Mouse (males) BALB/cBy	PCB77 10 or 100 mg/kg-d	Route: ip Duration: single dose; sac 4-5 d after SRBC injection	-	10 mg/kg-d	-	(Silkworth <i>et al.</i> , 1986)
Humoral immunity	PFC response (T-dependent; SRBC antigen)	Mouse (males) BALB/cBy donor; DBA/2 recipient	PCB77 10 or 100 mg/kg-d	Route: ip Duration: single dose; sac 4-5 d after SRBC injection	-	-	-	(Silkworth <i>et al.</i> , 1986)
Humoral immunity	PFC response (T-dependent; SRBC antigen)	Rabbit (both)	Aroclor 1248 [0.5, 4.0, or 13 mg/kg-d; estimated]	Route: oral (diet) Duration: 1/d; mated after 4w on diet, exposure continued through 4w of nursing when infants weaned and placed on normal diet. Tested @ 7w of age	13 mg/kg-d	-	-	(Thomas and Hinsdill, 1980)
Humoral immunity	PFC response (T-dependent; SRBC antigen) PFC response (Polyclonal; LPS antigen)	Mouse (males)	PCB206 4.6 to 46 mg/kg-d	Route: ip Duration: single dose; sac 4 d after immunization	12 mg/kg-d	46 mg/kg-d	-	(Harper <i>et al.</i> , 1993b)

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Category	Endpoint	Species (sex)	Test article Dose levels	Dosing parameters	NOAEL dose	LOAEL dose	Notes for NOAEL and/or LOAEL	Reference
Humoral immunity	PFC response (T-dependent; SRBC antigen) PFC response (Polyclonal; LPS antigen)	Mouse (males)	PCB207 4.6 to 46 mg/kg-d	Route: ip Duration: single dose; sac 4 d after immunization	46 mg/kg-d	-	-	(Harper <i>et al.</i> , 1993b)
Humoral immunity	PFC response (T-dependent; SRBC antigen) PFC response (Polyclonal; LPS antigen)	Mouse (males)	PCB208 4.6 to 46 mg/kg-d	Route: ip Duration: single dose; sac 4 d after immunization	46 mg/kg-d	-	-	(Harper <i>et al.</i> , 1993b)
Humoral immunity	PFC response (T-dependent; SRBC antigen) PFC response (Polyclonal; LPS antigen)	Mouse (males)	PCB209 5 to 50 mg/kg-d	Route: ip Duration: single dose; sac 4 d after immunization	50 mg/kg-d	-	-	(Harper <i>et al.</i> , 1993b)
Humoral immunity	PFC response (T-independent; DNP antigen)	Mouse (females exposed before mating; both sexes evaluated)	Kanechlor 500 14 mg/kg-d [divided wkly dose per day mg/kg-d; estimated]	Route: oral (gavage) Duration: 2/w; 3 w exposure to dams prior to mating, cross-over with controls during lactation to generate pre+postnatal, prenatal, or postnatal exposure grps. Offspring sac 5 d after DNP immunization	14 mg/kg-d	-	-	(Takagi <i>et al.</i> , 1987)

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Category	Endpoint	Species (sex)	Test article Dose levels	Dosing parameters	NOAEL dose	LOAEL dose	Notes for NOAEL and/or LOAEL	Reference
Humoral immunity	PFC response (T-independent; DNP antigen)	Mouse (females exposed before mating; both sexes evaluated)	Kanechlor 500 14 mg/kg-d [divided wkly dose per day mg/kg-d; estimated]	Route: oral (gavage) Duration: 2/w; 3 w exposure to dams prior to mating, cross-over with controls during lactation to generate pre+postnatal, prenatal, or postnatal exposure grps. Bacterial alpha-amylase (BaA) or DNP-KLH injections to mice generated BaA or DNP-KLH primed spleen cells for	14 mg/kg-d	-	-	(Takagi <i>et al.</i> , 1987)
Nonspecific immunity	Complement activity	Monkey (female)	Aroclor 1254 0.005 to 0.08 mg/kg-d	Route: oral (gelatin capsule) Duration: 1/d; 55 mo	-	0.005 mg/kg-d	-	(Tryphonas <i>et al.</i> , 1991b)
Nonspecific immunity	Macrophage killing assay	Mouse (males)	Aroclor 1242 [0.9 or 18 mg/kg-d; estimated]	Route: oral (diet) Duration: 1/d; 18w	0.9 mg/kg-d	18 mg/kg-d	-	(Loose <i>et al.</i> , 1981)
Nonspecific immunity	Macrophage killing assay	Mouse (males)	Aroclor 1242 [0.9 or 18 mg/kg-d; estimated]	Route: oral (diet) Duration: 1/d; 18w	18 mg/kg-d	-	-	(Loose <i>et al.</i> , 1981)
Nonspecific immunity	Macrophage killing assay	Mouse (males)	Aroclor 1242 [0.9 or 18 mg/kg-d; estimated]	Route: oral (diet) Duration: 1/d; 18w	18 mg/kg-d	-	-	(Loose <i>et al.</i> , 1981)
Nonspecific immunity	NK cell activity	Monkey (female)	Aroclor 1254 0.005 to 0.08 mg/kg-d	Route: oral (gelatin capsule) Duration: 1/d; 55 mo	0.08 mg/kg-d	-	-	(Tryphonas <i>et al.</i> , 1991b)

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Category	Endpoint	Species (sex)	Test article Dose levels	Dosing parameters	NOAEL dose	LOAEL dose	Notes for NOAEL and/or LOAEL	Reference
Nonspecific immunity	NK cell activity	Monkey (both)	Mix (major PCBs in Canadian human milk) 0.0075 mg/kg-d	Route: oral (formula) Duration: 1/d; 20 wks exposure, 66 wks total (w/ follow-up)	0.0075 mg/kg-d	-	-	(Arnold <i>et al.</i> , 1999)
Nonspecific immunity	NK cell activity	Monkey (males)	Mix (major PCBs in Canadian human milk) 0.0075 mg/kg-d	Route: oral (in corn oil) Duration: 1/d; 20 wks exposure, 66 wks total (w/ follow-up)	0.0075 mg/kg-d	-	-	(Arnold <i>et al.</i> , 1999)
Nonspecific immunity	NK cell activity	Mouse (both)	Fenclor 42 500 or 1,000 mg/kg-d	Route: ip Duration: 1/d; single dose	1000 mg/kg-d	-	-	(Franco <i>et al.</i> , 1989)
Nonspecific immunity	NK cell activity	Mouse (both)	Fenclor 42 500 or 1,000 mg/kg-d	Route: ip Duration: 1/d; 15d	1000 mg/kg-d	-	-	(Franco <i>et al.</i> , 1989)
Nonspecific immunity	NK cell activity	Mouse (females)	PCB118 82 to 260 mg/kg-d	Route: oral (gavage) Duration: single dose; sac 2 d after treatment	260 mg/kg-d	-	-	(Dahlman <i>et al.</i> , 1994)
Nonspecific immunity	NK cell activity	Mouse (females)	PCB77 7.3 to 29 mg/kg-d	Route: oral (gavage) Duration: single dose; sac 2 d after treatment	29 mg/kg-d	-	-	(Dahlman <i>et al.</i> , 1994)
Nonspecific immunity	NK cell activity	Rat (males)	Aroclor 1254 0.1 to 25 mg/kg-d	Route: oral (gavage) Duration: 1/d; 15w	1 mg/kg-d	10 mg/kg-d	-	(Smialowicz <i>et al.</i> , 1989)

Category	Endpoint	Species (sex)	Test article Dose levels	Dosing parameters	NOAEL dose	LOAEL dose	Notes for NOAEL and/or LOAEL	Reference
Nonspecific immunity	NK cell activity	Rat (males)	Aroclor 1254 [4.3 or 43 mg/kg-d; estimated]	Route: oral (diet) Duration: feed; 10 w	-	4.3 mg/kg-d	-	(Talcott <i>et al.</i> , 1985)
Nonspecific immunity	NK cell activity	Rat (males)	Aroclor 1254 [4.3 or 43 mg/kg-d; estimated]	Route: oral (diet) Duration: 1/d; 10 w	-	4.3 mg/kg-d	-	(Exon <i>et al.</i> , 1985)
Nonspecific immunity	NK cell activity	Rat (females)	Mix (derived from native herring oil) Pups cumulative intake at 11d (2.03, 19.9 ng TEQ/kg Atlantic, Baltic oils); at 25 d (1.83, 17.3 ng TEQ/kg Atlantic, Baltic oils) mg/kg-d	Route: oral (gavage) Duration: 1/d; GD6 to pup weaning (41 exposure days)	-	-	↓splenic NK cell activity in Baltic vs Atlantic group when results were corrected in include virus infection (ratio of 46 to 25 day old pups)	(Ross <i>et al.</i> , 1997)
Nonspecific immunity	NK cell activity	Rat (females)	Mix (derived from native herring) Atlantic: 0.3 ngTEQ/kg; Baltic: 1.6 ngTEQ/kg mg/kg-d	Route: oral (diet) Duration: 1/d; 4.5 mo	-	-	No change in splenic NK cell activity in Baltic vs Atlantic groups	(Ross <i>et al.</i> , 1996)

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Category	Endpoint	Species (sex)	Test article Dose levels	Dosing parameters	NOAEL dose	LOAEL dose	Notes for NOAEL and/or LOAEL	Reference
Nonspecific immunity	Phagocytotic activity	Monkey (females)	Aroclor 1254 0.04 to 0.08 mg/kg-d	Route: oral (gelatin capsule) Duration: 1/d; 55 mo	-	-	Dose-related ↓ trend toward reduced monocyte activation following stimulation with zymosan or PMA	(Tryphonas <i>et al.</i> , 1991a)
Nonspecific immunity	Phagocytotic activity	Mouse (males)	Aroclor 1242 [0.9 or 18 mg/kg-d; estimated]	Route: oral (diet) Duration: 1/d; 18w	18 mg/kg-d	-	-	(Loose <i>et al.</i> , 1981)
Nonspecific immunity	Phagocytotic activity	Mouse (both)	Aroclor 1254 0.167, 16.7 or 41.7 (dams) mg/kg-d	Route: oral (diet) Duration: 1/d; to 8 w of age	41.7 mg/kg-d	-	-	(Talcott and Koller, 1983)
Organ weight, histopath	Histology	Rabbit (males)	Aroclor 1254 0.18 to 6.54 mg/kg-d	Route: oral (diet) Duration: 1/d; 8 w	-	0.18 mg/kg-d	-	(Street and Sharma, 1975)
Organ weight, histopath	Organ weight	Mouse (males)	Aroclor 1016 [30 mg/kg-d; estimated]	Route: oral (diet) Duration: 1/d; 41w	30 mg/kg-d	-	-	(Silkworth and Loose, 1979a)
Organ weight, histopath	Organ weight	Mouse (both)	Fenclor 42 60 to 2,000 mg/kg-d	Route: ip Duration: 1/d; 15d	250 mg/kg-d	500 mg/kg-d	-	(Franco <i>et al.</i> , 1989)

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Category	Endpoint	Species (sex)	Test article Dose levels	Dosing parameters	NOAEL dose	LOAEL dose	Notes for NOAEL and/or LOAEL	Reference
Organ weight, histopath	Organ weight	Mouse (males)	PCB156	Route: ip	10 mg/kg-d	100 mg/kg-d	-	(Silkworth <i>et al.</i> , 1984)
			10 or 100 mg/kg-d	Duration: single dose; sac 5 d after SRBC injection				
	Organ weight	Mouse (males)	PCB156	Route: ip	100 mg/kg-d	-	-	(Silkworth <i>et al.</i> , 1984)
			10 or 100 mg/kg-d	Duration: single dose; sac 5 d after SRBC injection				
	Organ weight	Mouse (males)	PCB169	Route: oral (gavage)	1 mg/kg-d	5 mg/kg-d	-	(Kerkvliet and Baecher-Steppan, 1988)
			1 to 100 mg/kg-d	Duration: single dose; sac 12 d after P815				
	Organ weight	Mouse (females)	PCB169	Route: oral (gavage)	5 mg/kg-d	10 mg/kg-d	-	(Kerkvliet and Baecher-Steppan, 1988)
			1 to 100 mg/kg-d	Duration: single dose; sac 12 d after P815				
Organ weight, histopath	Organ weight	Mouse (males)	PCB169	Route: oral (gavage)	-	10 mg/kg-d	-	(Kerkvliet and Baecher-Steppan, 1988)
			10 or 100 mg/kg-d	Duration: single dose; sac 17-18 d after exposure				
Organ weight, histopath	Organ weight	Mouse (females)	PCB169	Route: oral (gavage)	10 mg/kg-d	100 mg/kg-d	-	(Kerkvliet and Baecher-Steppan, 1988)
			10 or 100 mg/kg-d	Duration: single dose; sac 17-18 d after exposure				
Organ weight, histopath	Organ weight	Mouse (males)	PCB47	Route: ip	100 mg/kg-d	-	-	(Silkworth <i>et al.</i> , 1984)
			10 or 100 mg/kg-d	Duration: single dose; sac 5 d after SRBC injection				

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Category	Endpoint	Species (sex)	Test article Dose levels	Dosing parameters	NOAEL dose	LOAEL dose	Notes for NOAEL and/or LOAEL	Reference
Organ weight, histopath	Organ weight	Mouse (males)	PCB47	Route: ip	100 mg/kg-d	-	-	(Silkworth <i>et al.</i> , 1984)
			10 or 100 mg/kg-d	Duration: single dose; sac 5 d after SRBC injection				
	Organ weight	Mouse (male) Ah(+)	PCB52	Route: ip	100 mg/kg-d	-	-	(Silkworth and Grabstein, 1982)
			10 or 100 mg/kg-d	Duration: 2 doses; sac 5 d after SRBC injection				
	Organ weight	Mouse (male) Ah(-)	PCB52	Route: ip	100 mg/kg-d	-	-	(Silkworth and Grabstein, 1982)
			10 or 100 mg/kg-d	Duration: 2 doses; sac 5 d after SRBC injection				
	Organ weight	Mouse (male) Ah(+)	PCB77	Route: ip	-	10 mg/kg-d	-	(Silkworth and Grabstein, 1982)
			10 or 100 mg/kg-d	Duration: 2 doses; sac 5 d after SRBC injection				
Organ weight, histopath	Organ weight	Mouse (male) Ah(-)	PCB77	Route: ip	100 mg/kg-d	-	-	(Silkworth and Grabstein, 1982)
			10 or 100 mg/kg-d	Duration: 2 doses; sac 5 d after SRBC injection				
Organ weight, histopath	Organ weight	Rat (males)	PCB105	Route: ip	-	-	-	(Safe <i>et al.</i> , 1989)
			Not specified mg/kg-d	Duration: single dose; sac 14d after treatment				
Organ weight, histopath	Organ weight	Rat (males)	PCB114	Route: ip	-	-	-	(Safe <i>et al.</i> , 1989)
			Not specified mg/kg-d	Duration: single dose; sac 14d after treatment				

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Category	Endpoint	Species (sex)	Test article Dose levels	Dosing parameters	NOAEL dose	LOAEL dose	Notes for NOAEL and/or LOAEL	Reference
Organ weight, histopath	Organ weight	Rat (males)	PCB114	Route: ip	-	-	-	(Safe <i>et al.</i> , 1989)
			Not specified mg/kg-d	Duration: single dose; sac 14d after treatment				
Organ weight, histopath	Organ weight	Rat (males)	PCB118	Route: ip	-	-	-	(Safe <i>et al.</i> , 1989)
			Not specified mg/kg-d	Duration: single dose; sac 14d after treatment				
Organ weight, histopath	Organ weight	Rat (males)	PCB126	Route: ip	-	-	-	(Safe <i>et al.</i> , 1989)
			Not specified mg/kg-d	Duration: single dose; sac 14d after treatment				
Organ weight, histopath	Organ weight	Rat (males)	PCB156	Route: ip	-	-	-	(Safe <i>et al.</i> , 1989)
			Not specified mg/kg-d	Duration: single dose; sac 14d after treatment				
Organ weight, histopath	Organ weight	Rat (males)	PCB157	Route: ip	-	-	-	(Safe <i>et al.</i> , 1989)
			Not specified mg/kg-d	Duration: single dose; sac 14d after treatment				
Organ weight, histopath	Organ weight	Rat (males)	PCB169	Route: ip	-	-	-	(Safe <i>et al.</i> , 1989)
			Not specified mg/kg-d	Duration: single dose; sac 14d after treatment				
Organ weight, histopath	Organ weight, histopath	Monkey (not specified [both])	Aroclor 1248	Route: in utero and nursing	0.1 mg/kg-d	0.2 mg/kg-d	-	(Allen <i>et al.</i> , 1980)
			0.1 or 0.2 mg/kg-d	Duration: 1/d; Discontinued treated diet of mothers 1 yr before mating				

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Category	Endpoint	Species (sex)	Test article Dose levels	Dosing parameters	NOAEL dose	LOAEL dose	Notes for NOAEL and/or LOAEL	Reference
Organ weight, histopath	Organ weight, histopath	Monkey (not specified [both])	Aroclor 1248 in utero mg/kg-d [0.1 or 0.2 mg/kg-d; estimated]	Route: in utero and nursing Duration: 1/d; 18 months (6 mo before breeding to control males, throughout gestation, and 3 mo after delivery). Infants placed on milk supplement at 4 mo of age	-	0.1 mg/kg-d	-	(Allen and Barsotti, 1976)
Organ weight, histopath	Organ weight, histopath	Monkey (both (1 mo old at study start))	Aroclor 1248 35 mg/kg-d	Route: oral (gavage) Duration: 1/d; 30 days	-	35 mg/kg-d	-	(Abrahamson and Allen, 1973)
Organ weight, histopath	Organ weight, histopath	Monkey (females)	Aroclor 1254 0.2 mg/kg-d	Route: oral (juice) Duration: 5/w; 12-13 mo	0.2 mg/kg-d	-	-	(Tryphonas <i>et al.</i> , 1986)
Organ weight, histopath	Organ weight, histopath	Monkey (females)	Aroclor 1254 0.2 mg/kg-d	Route: oral (juice) Duration: 5/w; 27-28 mo	0.2 mg/kg-d	-	-	(Tryphonas <i>et al.</i> , 1986)
Organ weight, histopath	Organ weight, histopath	Mouse (female)	Aroclor 1248 [20 or 200 mg/kg-d; estimated]	Route: oral (diet) Duration: 1/d; 5 w	200 mg/kg-d	-	-	(Thomas and Hinsdill, 1978)
Organ weight, histopath	Organ weight, histopath	Mouse (pups (both))	Aroclor 1254 [21 mg/kg-d; estimated]	Route: sc Duration: dams (3/wk); dams (2 w)	-	21 mg/kg-d	-	(Arena <i>et al.</i> , 2003)
Organ weight, histopath	Organ weight, histopath	Mouse (male)	Aroclor 1254 63 to 550 mg/kg-d	Route: ip Duration: single dose; injection 1 w before sac	-	63 mg/kg-d	-	(Wierda <i>et al.</i> , 1981)

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Category	Endpoint	Species (sex)	Test article Dose levels	Dosing parameters	NOAEL dose	LOAEL dose	Notes for NOAEL and/or LOAEL	Reference
Organ weight, histopath	Organ weight, histopath	Mouse (dams exposed, both sex of pups)	Aroclor 1254	Route: in utero (ip to dams prior to mating)	-	300 mg/kg-d	-	(Wu <i>et al.</i> , 1999)
			300 mg/kg-d	Duration: single dose; in utero/lactation exposure. Pups weaned at 22 d of age. Sac at 6 w of age.				
	Organ weight, histopath	Mouse (dams exposed, both sex of pups)	Aroclor 1254	Route: in utero (ip to dams prior to mating)	-	300 mg/kg-d	-	(Wu <i>et al.</i> , 1999)
			300 mg/kg-d	Duration: single dose; in utero/lactation exposure. Pups not weaned. Sac at 4 w of age.				
	Organ weight, histopath	Mouse (both)	Fenclor 42	Route: ip	-	500 mg/kg-d	-	(Franco <i>et al.</i> , 1989)
			500 or 1,000 mg/kg-d	Duration: 1/d; single dose				
Organ weight, histopath	Organ weight, histopath	Mouse (both)	Fenclor 42	Route: ip	-	500 mg/kg-d	-	(Franco <i>et al.</i> , 1989)
			500 or 1,000 mg/kg-d	Duration: 1/d; 15d				
	Organ weight, histopath	Mouse (both (pooled))	Mix (Aroclor 1242:Aroclor 1254 2:1)	Route: oral (diet)	-	4.7 mg/kg-d	-	(Segre <i>et al.</i> , 2002)
			[4.7 mg/kg-d; estimated]	Duration: 1/d; to 22 w of age				
Organ weight, histopath	Organ weight, histopath	Mouse (female)	PCB153	Route: oral (diet)	-	0.195 mg/kg-d	-	(Maranghi <i>et al.</i> , 2013)
			0.195 mg/kg-d	Duration: 1/d; 28d				

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Category	Endpoint	Species (sex)	Test article Dose levels	Dosing parameters	NOAEL dose	LOAEL dose	Notes for NOAEL and/or LOAEL	Reference
Organ weight, histopath	Organ weight, histopath	Mouse (males) BALB/cBy	PCB77 10 or 100 mg/kg-d	Route: ip Duration: single dose; sac 4-5 d after SRBC injection	10 mg/kg-d	100 mg/kg-d	-	(Silkworth <i>et al.</i> , 1986)
Organ weight, histopath	Organ weight, histopath	Mouse (males) CByD2F1	PCB77 10 or 100 mg/kg-d	Route: ip Duration: single dose; sac 4-5 d after SRBC injection	100 mg/kg-d	-	-	(Silkworth <i>et al.</i> , 1986)
Organ weight, histopath	Organ weight, histopath	Mouse (males) DBA/2	PCB77 10 or 100 mg/kg-d	Route: ip Duration: single dose; sac 4-5 d after SRBC injection	100 mg/kg-d	-	-	(Silkworth <i>et al.</i> , 1986)
Organ weight, histopath	Organ weight, histopath	Mouse (males) DBA/2 donor; BALB/cBy recipient	PCB77 10 or 100 mg/kg-d	Route: ip Duration: single dose; sac 4-5 d after SRBC injection	100 mg/kg-d	-	-	(Silkworth <i>et al.</i> , 1986)
Organ weight, histopath	Organ weight, histopath	Mouse (males) DBA/2 donor & recipient	PCB77 10 or 100 mg/kg-d	Route: ip Duration: single dose; sac 4-5 d after SRBC injection	100 mg/kg-d	-	-	(Silkworth <i>et al.</i> , 1986)
Organ weight, histopath	Organ weight, histopath	Mouse (males) BALB/cBy donor & recipient	PCB77 10 or 100 mg/kg-d	Route: ip Duration: single dose; sac 4-5 d after SRBC injection	-	10 mg/kg-d	-	(Silkworth <i>et al.</i> , 1986)

Category	Endpoint	Species (sex)	Test article Dose levels	Dosing parameters	NOAEL dose	LOAEL dose	Notes for NOAEL and/or LOAEL	Reference
471	Organ weight, histopath	Mouse (males) BALB/cBy donor; DBA/2 recipient	PCB77 10 or 100 mg/kg-d	Route: ip Duration: single dose; sac 4-5 d after SRBC injection	-	10 mg/kg-d	-	(Silkworth <i>et al.</i> , 1986)
	Organ weight, histopath	Rabbit (both)	Aroclor 1248 [0.5, 4.0, or 13 mg/kg-d; estimated]	Route: oral (diet) Duration: 1/d; mated after 4w on diet, exposure continued through 4w of nursing when infants weaned and placed on normal diet. Tested @ 7w of age	13 mg/kg-d	-	-	(Thomas and Hinsdill, 1980)
	Organ weight, histopath	Rabbit (females)	Aroclor 1260 [42 mg/kg-d; estimated]	Route: dermal Duration: 1/day, 5d/w; 38d (27 treatments)	-	42 mg/kg-d	-	(Vos and Beems, 1971)
	Organ weight, histopath	Rabbit (females)	Aroclor 1260 [44 mg/kg-d; estimated]	Route: dermal Duration: 1/day, 5d/w; 28 d (20 treatments)	-	44 mg/kg-d	-	(Vos and Notenboom-Ram, 1972)
	Organ weight, histopath	Rabbit (females)	Clophen A60 [42 mg/kg-d; estimated]	Route: dermal Duration: 1/day, 5d/w; 38d (27 treatments)	-	42 mg/kg-d	-	(Vos and Beems, 1971)
	Organ weight, histopath	Rabbit (females)	PCB153 [44 mg/kg-d; estimated]	Route: dermal Duration: 1/day, 5d/w; 28 d (20 treatments)	-	44 mg/kg-d	-	(Vos and Notenboom-Ram, 1972)

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Category	Endpoint	Species (sex)	Test article Dose levels	Dosing parameters	NOAEL dose	LOAEL dose	Notes for NOAEL and/or LOAEL	Reference
Organ weight, histopath	Organ weight, histopath	Rabbit (females)	Phenoclor DP6 [42 mg/kg-d; estimated]	Route: dermal Duration: 1/day, 5d/w; 38d (27 treatments)	-	42 mg/kg-d	-	(Vos and Beems, 1971)
Organ weight, histopath	Organ weight, histopath	Rat (males)	PCB128 0.0042, 0.042, 0.43, 4.2 mg/kg-d	Route: oral (diet) Duration: 1/d; 13 w	4.2 mg/kg-d	-	-	(Lecavalier <i>et al.</i> , 1997)
Organ weight, histopath	Organ weight, histopath	Rat (females)	PCB128 0.0045, 0.045, 0.44, 4.4 mg/kg-d	Route: oral (diet) Duration: 1/d; 13 w	4.4 mg/kg-d	-	-	(Lecavalier <i>et al.</i> , 1997)
Organ weight, histopath	Organ weight, histopath, cellularity	Mouse (pups (both))	Aroclor 1242 [21 mg/kg-d; estimated]	Route: sc Duration: dams (3/wk); dams (2 w)	-	21 mg/kg-d	Thymus cellularity changes at 7 days of age; no difference by 28 days. No changes to spleen cellularity	(Arena <i>et al.</i> , 2003)
Organ weight, histopath	Organ weight, histopath, cellularity	Mouse (female)	PCB126 0.2 mg/kg-d	Route: oral (gavage) Duration: single dose; sac 4d after treatment	-	0.2 mg/kg-d	-	(Pan <i>et al.</i> , 2004)
Organ weight, histopath	Organ weight, histopath, cellularity	Mouse (female)	PCB169 2 or 5 mg/kg-d	Route: oral (gavage) Duration: single dose; sac 4d after treatment	2 mg/kg-d	5 mg/kg-d	-	(Pan <i>et al.</i> , 2004)

Category	Endpoint	Species (sex)	Test article Dose levels	Dosing parameters	NOAEL dose	LOAEL dose	Notes for NOAEL and/or LOAEL	Reference
Organ weight, histopath	Organ weight, histopath, cellularity	Mouse (female)	PCB169 2 or 5 mg/kg-d	Route: oral (gavage) Duration: single dose; sac 4d after treatment	2 mg/kg-d	5 mg/kg-d	-	(Pan <i>et al.</i> , 2004)
Organ weight, histopath	Organ weight, histopath	Mouse (both)	PCB126 0.0005 to 0.5 mg/kg-d	Route: oral (gavage) Duration: 1/d; up to 7 d	-	0.005 mg/kg	-	(Du <i>et al.</i> , 2019)
Other	2-year chronic immunotoxicity endpoints	Rat (females)	PCB118 0.1, 0.22, 0.46, 1.0, or 4.6 mg/kg-d	Route: oral (gavage) Duration: 5d/w; 105 w	1 mg/kg-d	4.6 mg/kg-d	Bone marrow hyperplasia	(NTP, 2010)
Other	2-year chronic immunotoxicity endpoints	Rat (females)	PCB126 0.000030, 0.000100, 0.000175, 0.000300, 0.000550, or 0.0010 mg/kg-d	Route: oral (gavage) Duration: 5d/w; 105 w	0.000100 mg/kg-d	0.000175 mg/kg-d	Thymic atrophy	(NTP, 2006c)
Other	2-year chronic immunotoxicity endpoints	Rat (females)	PCB153 0.01, 0.1, 0.3, 1.0, or 3.0 mg/kg-d	Route: oral (gavage) Duration: 5d/w; 105 w	1.00 mg/kg-d	3.00 mg/kg-d	Bone marrow hyperplasia	(NTP, 2006a)
Other	Diabetes development	Mouse (females)	PCB153 0.125 or 12.5 mg/kg-d	Route: ip Duration: bi-weekly; up to 16 w (or until mouse became diabetic)	-	0.125 mg/kg-d	-	(Kuiper <i>et al.</i> , 2016)

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Category	Endpoint	Species (sex)	Test article Dose levels	Dosing parameters	NOAEL dose	LOAEL dose	Notes for NOAEL and/or LOAEL	Reference
Other	Globulin producing cells, Popliteal LN	Rabbit (males)	Aroclor 1254 0.18 to 6.54 mg/kg-d	Route: oral (diet) Duration: 1/d; 8 w	6.54 mg/kg-d	-	-	(Street and Sharma, 1975)
Other	Gut microbiome	Mouse (male)	Mix (PCB138, PCB153, PCB180 1.7:3.2:1, molar ratio) 55 (16, 29, 10 of PCB138, 153, 180) mg/kg-d	Route: oral (gavage) Duration: single dose; 2d post exposure	-	55 mg/kg-d	-	(Choi <i>et al.</i> , 2013)
Other	IL-1 release, monocytes +LPS	Monkey (females)	Aroclor 1254 0.005 to 0.08 mg/kg-d	Route: oral (gelatin capsule) Duration: 1/d; 55 mo	0.08 mg/kg-d	-	Trend for ↓IL1 release following <i>in vitro</i> stimulation of monocytes with LPS (no group was statistically different than control).	(Tryphonas <i>et al.</i> , 1991a)
Other	IL-2 release, splenocytes, bkg levels following KLH injections	Rat (males)	Aroclor 1254 [4.3 or 43 mg/kg-d; estimated]	Route: oral (diet) Duration: 1/d; 10 w	-	4.3 mg/kg-d	-	(Exon <i>et al.</i> , 1985)
Other	IL-5 release, splenocytes cultured +OVA	Mouse (female)	PCB126 0.2 mg/kg-d	Route: oral (gavage) Duration: single dose; sac 4d after treatment	-	0.2 mg/kg-d	-	(Pan <i>et al.</i> , 2004)

Category	Endpoint	Species (sex)	Test article Dose levels	Dosing parameters	NOAEL dose	LOAEL dose	Notes for NOAEL and/or LOAEL	Reference
Other	IL-5 release, splenocytes cultured +OVA	Mouse (female)	PCB169 2 or 5 mg/kg-d	Route: oral (gavage) Duration: single dose; sac 4d after treatment	5 mg/kg-d	-	-	(Pan <i>et al.</i> , 2004)
Other	Liver lesions with MLV infection	Mouse (males)	Aroclor 1221 [0.7, 7, 70 mg/kg-d; Duration: 1/d; 6 mo estimated]	Route: oral (diet)	70 mg/kg-d	-	-	(Koller, 1977)
Other	Liver lesions with MLV infection	Mouse (males)	Aroclor 1242 [0.7, 7, 70 mg/kg-d; Duration: 1/d; 6 mo estimated]	Route: oral (diet)	-	0.7 mg/kg-d	-	(Koller, 1977)
Other	Liver lesions with MLV infection	Mouse (males)	Aroclor 1254 [0.7, 7, 70 mg/kg-d; Duration: 1/d; 6 mo estimated]	Route: oral (diet)	-	0.7 mg/kg-d	-	(Koller, 1977)
Other	Oxygen consumption (macrophages, PMNs, dead yeast)	Mouse (males)	Aroclor 1242 [0.9 or 18 mg/kg-d; Duration: 1/d; 18w estimated]	Route: oral (diet)	18 mg/kg-d	-	-	(Loose <i>et al.</i> , 1981)
Other	Peritoneal cell population following P815 injection	Mouse (female)	PCB169 10 mg/kg-d	Route: oral (gavage) Duration: single dose; sac 6-10 d after P815	-	10 mg/kg- d	-	(Kerkvliet and Baecher- Steppan, 1988)
Other	PGE2 release following P815 injection	Mouse (male)	PCB169 10 mg/kg-d	Route: oral (gavage) Duration: single dose; sac 9d post P815 injection	-	10 mg/kg- d	-	(De Krey <i>et al.</i> , 1994a)

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Category	Endpoint	Species (sex)	Test article Dose levels	Dosing parameters	NOAEL dose	LOAEL dose	Notes for NOAEL and/or LOAEL	Reference
476	Other	Potential of endometriosis lesion	Mouse (females) PCB126 0.1 to 1 mg/kg-d	Route: oral (gavage) Duration: 3 w btwn doses for 5 total doses; 16 w	1 mg/kg-d	-	-	(Johnson <i>et al.</i> , 1997)
	Other	Potential of endometriosis lesion	Mouse (females) PCB153 3 to 30 mg/kg-d	Route: oral (gavage) Duration: 3 w btwn doses for 5 total doses; 16 w	30 mg/kg-d	-	-	(Johnson <i>et al.</i> , 1997)
	Other	Reporting clinical findings prior to breeding portion of on-going study	Monkey (females) Aroclor 1254 0.005 to 0.08 mg/kg-d	Route: oral (gelatin capsule) Duration: 1/d; 37 mo	-	-	-	(Arnold <i>et al.</i> , 1993a)
	Other	Serum interferon inducibility	Mouse (males) Kanechlor 500 18, 33, or 66 mg/kg-d	Route: oral (diet) Duration: daily; 21 d	66 mg/kg-d	-	-	(Imanishi <i>et al.</i> , 1980)
	Other	Reactive oxygen species (SOD/MDA in spleen, thymus supernatant)	Mouse (both) PCB126 0.0005 to 0.5 mg/kg-d	Route: oral (gavage) Duration: 1/d; up to 7 d	0.0005 mg/kg	0.005 mg/kg	-	(Du <i>et al.</i> , 2019)
	Other	Reactive oxygen species (DCFH-DA intracellular probe in plated spleen cells)	Mouse (both) PCB126 0.0005 to 0.5 mg/kg-d	Route: oral (gavage) Duration: 1/d; up to 7 d	-	0.0005 mg/kg	-	(Du <i>et al.</i> , 2019)
	Resistance	Ectromelia virus	Mouse (males) Kanechlor 500 18, 33, or 66 mg/kg-d	Route: oral (diet) Duration: daily; 21 d	18 mg/kg-d	33 mg/kg-d	-	(Imanishi <i>et al.</i> , 1980)

Category	Endpoint	Species (sex)	Test article Dose levels	Dosing parameters	NOAEL dose	LOAEL dose	Notes for NOAEL and/or LOAEL	Reference
Resistance	EL-4 tumor cell injection	Mouse (males)	Aroclor 1242 [0.9 or 18 mg/kg-d; estimated]	Route: oral (diet) Duration: 1/d; 18w	0.9 mg/kg-d	18 mg/kg-d	-	(Loose <i>et al.</i> , 1981)
Resistance	Endotoxin	Mouse (males)	Aroclor 1016 [1 mg/kg-d; estimated]	Route: oral (diet) Duration: 1/d; 3w	-	1 mg/kg-d	-	(Loose <i>et al.</i> , 1978a) (Loose <i>et al.</i> , 1977) (Loose <i>et al.</i> , 1979)
Resistance	Endotoxin	Mouse (males)	Aroclor 1242 [1 mg/kg-d; estimated]	Route: oral (diet) Duration: 1/d; 3w	-	1 mg/kg-d	-	(Loose <i>et al.</i> , 1978a) (Loose <i>et al.</i> , 1977) (Loose <i>et al.</i> , 1979)
Resistance	Endotoxin	Mouse (males)	Aroclor 1242 [30 mg/kg-d; estimated]	Route: oral (diet) Duration: 1/d; 6w	-	30 mg/kg-d	-	(Loose <i>et al.</i> , 1978b)
Resistance	Endotoxin	Mouse (female)	Aroclor 1248 [20 or 200 mg/kg-d; estimated]	Route: oral (diet) Duration: 1/d; 5 w	-	20 mg/kg-d	-	(Thomas and Hinsdill, 1978)
Resistance	Herpes simplex virus [HSV]	Mouse (males)	Kanechlor 500 18, 33, or 66 mg/kg-d	Route: oral (diet) Duration: daily; 21 d	18 mg/kg-d	33 mg/kg-d	-	(Imanishi <i>et al.</i> , 1980)
Resistance	L1210 tumor cells	Mouse (males)	Aroclor 1242 [0.9 or 18 mg/kg-d; estimated]	Route: oral (diet) Duration: 1/d; 18w	18 mg/kg-d	-	-	(Loose <i>et al.</i> , 1981)

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Category	Endpoint	Species (sex)	Test article Dose levels	Dosing parameters	NOAEL dose	LOAEL dose	Notes for NOAEL and/or LOAEL	Reference
Resistance	Listeria, HSV-2	Mouse (not specified)	Aroclor 1254 30 mg/kg-d	Route: oral (gavage) Duration: 1/d; 14 days prior to i.v. injection of microorganism	30 mg/kg-d	-	-	(Bradley and Morahan, 1982)
Resistance	Listeria monocytogenes	Mouse (not specified)	Aroclor 1254 500 mg/kg-d	Route: ip Duration: single dose; deaths recorded daily	-	500 mg/kg-d	-	(Lubet <i>et al.</i> , 1986)
Resistance	Malaria	Mouse (males)	Aroclor 1016 [1 mg/kg-d; estimated]	Route: oral (diet) Duration: 1/d; 6w	1 mg/kg-d	-	-	(Loose <i>et al.</i> , 1978a) (Loose <i>et al.</i> , 1977) (Loose <i>et al.</i> , 1979)
Resistance	Malaria	Mouse (males)	Aroclor 1242 [1 mg/kg-d; estimated]	Route: oral (diet) Duration: 1/d; 6w	1 mg/kg-d	-	-	(Loose <i>et al.</i> , 1978a) (Loose <i>et al.</i> , 1977) (Loose <i>et al.</i> , 1979)
Resistance	Malaria	Mouse (males)	Aroclor 1242 [30 mg/kg-d; estimated]	Route: oral (diet) Duration: 1/d; 6w	-	30 mg/kg- d	-	(Loose <i>et al.</i> , 1978a; Loose <i>et al.</i> , 1978b)
Resistance	mKSA tumor cell injection	Mouse (males)	Aroclor 1242 [0.9 or 18 mg/kg-d; estimated]	Route: oral (diet) Duration: 1/d; 18w	18 mg/kg-d	-	-	(Loose <i>et al.</i> , 1981)
Resistance	P388 tumor cells	Mouse (males)	Aroclor 1242 [0.9 or 18 mg/kg-d; estimated]	Route: oral (diet) Duration: 1/d; 18w	18 mg/kg-d	-	-	(Loose <i>et al.</i> , 1981)

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Category	Endpoint	Species (sex)	Test article Dose levels	Dosing parameters	NOAEL dose	LOAEL dose	Notes for NOAEL and/or LOAEL	Reference
Resistance	PYB6 fibrosarcoma	Mouse (not specified)	Aroclor 1254 500 mg/kg-d	Route: ip Duration: single dose; examined 2x/w for tumor appearance	-	500 mg/kg-d	-	(Lubet <i>et al.</i> , 1986)
Resistance	RMCV	Rat (females)	Mix (derived from native herring oil) Pups cumulative intake at 11d (2.03, 19.9 ng TEQ/kg Atlantic, Baltic oils); at 25 d (1.83, 17.3 ng TEQ/kg Atlantic, Baltic oils) mg/kg-d	Route: oral (gavage) Duration: 1/d; GD6 to pup weaning (41 exposure days)	-	-	RCMV-specific (IgG) titres of Baltic-fed infected rats were lower than those in Atlantic group at 25d post-infection. RCMV (virus) titres were not different	(Ross <i>et al.</i> , 1997)
Resistance	RMCV	Rat (females)	Mix (derived from native herring) Atlantic: 0.3 ngTEQ/kg; Baltic: 1.6 ngTEQ/kg mg/kg-d	Route: oral (diet) Duration: 1/d; 4.5 mo	-	-	No difference in RCMV-specific (IgG or IgM) titres. RCMV (virus) titres of Baltic-fed infected rats were higher than those in Atlantic group	(Ross <i>et al.</i> , 1996)
Resistance	Salmonella typhimurium	Mouse (female)	Aroclor 1248 [200 mg/kg-d; estimated]	Route: oral (diet) Duration: 1/d; 5 w	-	200 mg/kg-d	-	(Thomas and Hinsdill, 1978)

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Category	Endpoint	Species (sex)	Test article Dose levels	Dosing parameters	NOAEL dose	LOAEL dose	Notes for NOAEL and/or LOAEL	Reference
Resistance	Tetanus toxin	Guinea pig (females)	Aroclor 1260 [6 mg/kg-d; estimated]	Route: oral (diet) Duration: 1/d; 6 w	6 mg/kg-d	-	-	(Vos and van Driel-Grootenhuys, 1972)
Resistance	Tetanus toxin	Guinea pig (females)	Aroclor 1260 [1 or 5 mg/kg-d; estimated]	Route: oral (diet) Duration: 1/d; 8 w	-	1 mg/kg-d	-	(Vos and de Roij, 1972)
Resistance	Tetanus toxin	Guinea pig (males)	Clophen A60 [0.94, 4.7, or 24 mg/kg-d; estimated]	Route: oral (diet) Duration: 1/d; 3w	0.94 mg/kg-d	4.7 mg/kg-d	-	(Vos and van Driel-Grootenhuys, 1972)
Resistance	Tetanus toxin	Guinea pig (females)	Clophen A60 [1 or 6 mg/kg-d; estimated]	Route: oral (diet) Duration: 1/d; 6 w	1 mg/kg-d	6 mg/kg-d	-	(Vos and van Driel-Grootenhuys, 1972)

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D. Attachment 4. Animal studies included in evaluation of potential neurodevelopmental effects of PCBs

**LIST OF ANIMAL STUDIES INCLUDED IN EVALUATION OF NEURODEVELOPMENTAL EFFECTS OF PCBs
(ONLY STUDIES ADMINISTERING ORAL AND REPEATED DOSES WERE CONSIDERED)**

Internal Study ID	Species	Congener	Dose	Units	Reference
NA002	Rat	Aroclor 1254	0 or 15	mg/kg-d	(Bowers <i>et al.</i> , 2004)
NA003	NHP	Aroclor 1248	0, 2.5 or 5.0	ppm feed	(Bowman <i>et al.</i> , 1978)
NA004	NHP	Aroclor 1248	0, 2.5	ppm feed	(Bowman and Heironimus, 1981)
NA005	Mouse	Aroclor 1254	0 or 10	mg/kg-d	(Branchi <i>et al.</i> , 2005)
NA006	Rat	Aroclor 1254	0, 1 or 6	mg/kg-d	(Bushnell <i>et al.</i> , 2002)
NA007	Rat	Unspecified mixture in contaminated fish	0 or 0.72	µg/g feed	(Carpenter <i>et al.</i> , 2002)
NA009	Rat	PCB126 or PCB153	0 or 0.0001 (PCB126); 0 or 1 (PCB153)	mg/kg-d	(Cauli <i>et al.</i> , 2013)
NA022	Rat	Mixture of PCB47 and PCB77	0, 1.25, 12.5 or 25	ppm feed	(Donahue <i>et al.</i> , 2004)
NA024	Mouse	Mixture of PCB28, 52, 101, 138, 153 and 180	0, 0.000001, 0.00001 or 0.0001	mg/kg-d	(Elnar <i>et al.</i> , 2012)
NA030	Rat	Aroclor 1254	0 or 8	mg/kg-d	(Goldey and Crofton, 1998)

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Internal Study ID	Species	Congener	Dose	Units	Reference
NA033	Mouse	PCB153	Gestation: 0.00073 or 0.170 (casein); 0.00069 or 0.160 (fish); Lactation: 0.00172 or 0.350 (casein); 0.00163 or 0.420 (fish)	mg/kg-d	(Haave <i>et al.</i> , 2011)
NA035	Rat	PCB118 or PCB126	0, 1 or 5 (PCB118); 0 or 0.002 (PCB126)	mg/kg-d	(Holene <i>et al.</i> , 1995)
NA036	Rat	PCB153 or PCB126	0 or 5 (PCB153); 0 or 0.002 (PCB126)	mg/kg-d	(Holene <i>et al.</i> , 1998)
NA037	Rat	PCB153	0 or 5	mg/kg-d	(Holene <i>et al.</i> , 1999)
NA039	Rat	Mixture of PCB47 and 77	0, 12.5 or 25	ppm feed	(Jolous-Jamshidi <i>et al.</i> , 2010)
NA042	Rat	Aroclor 1254	0 or 6	mg/kg-d	(Lasky <i>et al.</i> , 2002)
NA043	NHP	Aroclor 1248	0 or 2.5	ppm feed	(Levin <i>et al.</i> , 1988)
NA044	Rat	Chlophen A30	0 or 30	mg/kg-d	(Lilienthal and Winneke, 1991)
NA045	Rat	Chlophen A30	0, 5 or 30	mg/kg -d	(Lilienthal <i>et al.</i> , 1990)
NA048	NHP	Aroclor 1248	0, 0.5 or 2.5	ppm feed	(Mele <i>et al.</i> , 1986)
NA050	Rat	Aroclor 1254	0 or 10	mg/kg-d	(Nguon <i>et al.</i> , 2005)
NA052	Rat	Aroclor 1254	0.02 (control), 2.5, 26 or 269	ppm feed	(Overmann <i>et al.</i> , 1987)
NA056	NHP	Mixture of PCB52, 66, 74, 105, 118, 138, 153, 156, 157, 180, 183, 187, 189, 194 and 203)	0 or 0.0075	mg/kg-d	(Rice, 1997)

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Internal Study ID	Species	Congener	Dose	Units	Reference
NA057	NHP	Mixture of PCB52, 66, 74, 105, 118, 138, 153, 156, 157, 180, 183, 187, 189, 194 and 203)	0 or 0.0075	mg/kg-d	(Rice, 1998)
NA059	NHP	Mixture of PCB52, 66, 74, 105, 118, 138, 153, 156, 157, 180, 183, 187, 189, 194 and 203)	0 or 0.0075	mg/kg-d	(Rice and Hayward, 1997)
NA060	NHP	Mixture of PCB52, 66, 74, 105, 118, 138, 153, 156, 157, 180, 183, 187, 189, 194 and 203)	0 or 0.0075	mg/kg-d	(Rice and Hayward, 1999b)
NA061	Rat	PCB126	0, 0.00025 or 0.001	mg/kg-d	(Rice, 1999)
NA062	Rat	PCB126	0, 0.00025 or 0.001	mg/kg-d	(Rice and Hayward, 1998)
NA063	Rat	Aroclor 1254	0, 6	mg/kg-d	(Roegge <i>et al.</i> , 2000)
NA064	Rat	Aroclor 1254	0 or 6	mg/kg-d	(Roegge <i>et al.</i> , 2004)
NA065	Rat	Mixture of Aroclor 1242, 1248, 1254, and 1260	0, 1, 3 or 6	mg/kg-d	(Sable <i>et al.</i> , 2006)
NA066	NHP	Aroclor 1248	0, 2.5	ppm feed	(Schantz <i>et al.</i> , 1989)
NA067	Rat	28, 118, 153	0, 8 or 32	mg/kg-d	(Schantz <i>et al.</i> , 1995)

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Internal Study ID	Species	Congener	Dose	Units	Reference
NA069	Mouse	Aroclor 1254	0, 6, 18 or 54	mg/kg-d	(Sugawara <i>et al.</i> , 2006)
NA070	Rat	Aroclor 1254	0, 6	mg/kg-d	(Taylor <i>et al.</i> , 2002)
NA071	Rat	Aroclor 1254	0, 6	mg/kg-d	(Widholm <i>et al.</i> , 2001)
NA072	Rat	Aroclor 1254	0 or 6	mg/kg-d	(Widholm <i>et al.</i> , 2004)
NA074	Rat	Aroclor 1254	0, 1 or 6	mg/kg-d	(Yang <i>et al.</i> , 2009)
NA075	Rat	Aroclor 1016 or Aroclor 1254	0 or 10 (Aroclor 1016); 0 or 8 (Aroclor 1254)	mg/kg-d	(Zahalka <i>et al.</i> , 2001)
NA076	Mouse	PCB 77	0 or 32	mg/kg-d	(Agrawal <i>et al.</i> , 1981)
NA077	Rat	PCB52, 138 or 180	0 or 1	mg/kg-d	(Boix <i>et al.</i> , 2010)
NA078	Rat	PCB126	0, 0.25 or 1	mg/kg-d	(Bushnell and Rice, 1999)
NA079	Mouse	PCB77	0 or 32	mg/kg-d	(Chou <i>et al.</i> , 1979)
NA081	Mouse	Mixture of PCB28, 52, 101, 138, 153 and 180	0 or 0.00001	mg/kg-d	(Elnar <i>et al.</i> , 2016)
NA082	Rat	Aroclor 1254	0, 1 or 6	mg/kg-d	(Geller <i>et al.</i> , 2001)
NA086	Rat	PCB52, PCB180 or PCB153	0 or 10	mg/kg-d	(Johansen <i>et al.</i> , 2011)
NA089	Rat	Aroclor 1254	0 or 25	mg/kg-d	(Meerts <i>et al.</i> , 2004)
NA091	Rat	PCB153 or PCB126	0 or 1 (PCB153); 0 or 0.0001 (PCB26)	mg/kg-d	(Piedrafita <i>et al.</i> , 2008)

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Internal Study ID	Species	Congener	Dose	Units	Reference
NA093	Rat	Mixture of Aroclors 1242, 1248, 1254, and 1260	0, 1, 3 or 6	mg/kg-d	(Poon <i>et al.</i> , 2015)
NA094	Rat	Aroclor 1254	0, 1.25 or 12.5	ppm feed	(Provost <i>et al.</i> , 1999)
NA096	Rat	PCB126	0, 0.00025 or 0.001	mg/kg-d	(Rice and Hayward, 1999a)
NA097	Rat	Mixture of Aroclor 1242, 1248, 1254 and 1260	0, 1 or 3	mg/kg-d	(Sable <i>et al.</i> , 2009)
NA098	Rat	PCB77 or PCB126	0, 2 or 8 (PCB77); 0, 0.00025 or 0.001 (PCB126)	mg/kg-d	(Schantz <i>et al.</i> , 1996)
NA099	Rat	PCB95	0, 8 or 32	mg/kg-d	(Schantz <i>et al.</i> , 1997)
NA100	Rat	Kanechlor 500	0, 20 or 100	mg/kg-d	(Shiota, 1976)
NA101	Rat	PCB153	0, 1 or 5	mg/kg-d	(Sitarek and Gralawicz, 2009)
NA102	Mouse	Aroclor 1254	0 or 18	mg/kg-d	(Sugawara <i>et al.</i> , 2008)
NA103	Mouse	Aroclor 1254	0, 6 or 18	mg/kg-d	(Tian <i>et al.</i> , 2011)
NA104	Mouse	PCB77	0 or 32	mg/kg-d	(Tilson <i>et al.</i> , 1979)
NA105	Rat	PCB126	0 or 0.0001	mg/kg-d	(Vitalone <i>et al.</i> , 2008)
NA106	Rat	PCB126	0 or 0.0001	mg/kg-d	(Vitalone <i>et al.</i> , 2010)
NA108	Rat	Fenclor 42	0, 2 or 4	mg/kg-d	(Pantaleoni <i>et al.</i> , 1988)
NA109	NHP	Aroclor 1248	0 or 2.5	ppm feed	(Bowman and Heironimus, 1981)

Internal Study ID	Species	Congener	Dose	Units	Reference
NA110	Mouse	Mixture of PCB28, 52, 101, 138, 153, and 180	0, 0.000085, 0.000216 or 0.0004	mg/kg/d	(Dridi <i>et al.</i> , 2014)
NA111	Mouse	Mixture of PCB77, P105, P118, P126, P138, P153, P169 and P180	10 mg/kg each of PCB105, 118, 138, 153 and 180; 5 mg/kg (PCB77); 25 µg/kg (PCB126); 250 µg/kg-d (PCB169)	mg/kg-d or µg/kg-d	(Curran <i>et al.</i> , 2012)
NA115	Rat	PCB153	0, 1 or 5	mg/kg-d	(Gralewicz <i>et al.</i> , 2009)
NA117	Mouse	Aroclor 1254	0, 11 or 82	ppm feed	(Storm <i>et al.</i> , 1980)
NA118	Mouse	Mixture of PCB28, 52, 101, 138, 153 and 180	0, 0.00001 or 0.001	mg/kg-d	(Karkaba <i>et al.</i> , 2017)
NA119	Rat	PCB153	0, 1, 3 or 6	mg/kg-d	(Johansen <i>et al.</i> , 2014)
NA120	Rat	Mixture of Aroclor 1242, 1248, 1254 and 1260	0 or 6	mg/kg-d	(Bandara <i>et al.</i> , 2016)
NA121	Rat	P52, P138 or P180	0 or 1	mg/kg-d	(Boix <i>et al.</i> , 2011)
NA123	Rat	PCB126	0, 0.00025 or 0.001	mg/kg-d	(Geller <i>et al.</i> , 2001)
NA125	Mouse	Mixture of PCB77, P105, P118, P126, P138, P153, P169 and P180	10 mg/kg each of PCB105, 118, 138, 153 and 180; 5 mg/kg (PCB77); 25 µg/kg (PCB126); 250 µg/kg-d (PCB169)	mg/kg-d or µg/kg-d	(Colter <i>et al.</i> , 2018)
NA126	Rat	Aroclor 1254	0 or 6	mg/kg-d	(Crofton <i>et al.</i> , 2000b)

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Internal Study ID	Species	Congener	Dose	Units	Reference
NA127	Rat	Aroclor 1254	0 or 8	mg/kg-d	(Crofton <i>et al.</i> , 2000a)
NA128	Rat	Mixture of PCB47 and 77	0, 12.5 or 25	ppm feed	(Cromwell <i>et al.</i> , 2007)
NA130	Rat	Aroclor 1254	0, 1, 4 or 8	mg/kg-d	(Goldey <i>et al.</i> , 1995)
NA131	Rat	Mixture of PCB28, 77, 101, 105, 118, 126, 138, 146, 153, 156, 169, 170, 180 and 187 or Aroclor 1254	0 or 4	mg/kg-d	(Hany <i>et al.</i> , 1999)
NA132	Rat	Aroclor 1254	0, 1, 4 or 8	mg/kg-d	(Herr <i>et al.</i> , 1996)
NA133	Rat	Aroclor 1254	0, 1 or 6	mg/kg-d	(Herr <i>et al.</i> , 2001)
NA134	Rat	Mixture of PCB28, 77, 101, 105, 118, 126, 138, 146, 153, 156, 169, 170, 180 and 187	0, 0.5, 2 or 4	mg/kg-d	(Kaya <i>et al.</i> , 2002)
NA135	Rat	PCB95	0 or 6	mg/kg-d	(Kenet <i>et al.</i> , 2007)
NA137	Rat	PCB52 or PCB180	0, 3, 10, 30, 100 or 300	mg/kg-d	(Lilienthal <i>et al.</i> , 2011)
NA138	Rat	PCB74	0 or 11.68	mg/kg-d	(Lilienthal <i>et al.</i> , 2013)
NA139	Rat	Mixture of Aroclor 1242, 1248, 1254 and 1260	0, 3 or 6	mg/kg-d	(Poon <i>et al.</i> , 2011)

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Internal Study ID	Species	Congener	Dose	Units	Reference
NA140	Rat	Mixture of Aroclor 1242, 1248, 1254 and 1260	0, 1, 3 or 6	mg/kg-d	(Powers <i>et al.</i> , 2006)
NA141	Rat	Mixture of Aroclor 1242, 1248, 1254 and 1260	0, 1 or 3	mg/kg-d	(Powers <i>et al.</i> , 2009)

NHP, nonhuman primate

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E. Attachment 5. Animal studies included in evaluation of potential reproductive effects of PCBs

LIST OF ANIMAL STUDIES INCLUDED IN EVALUATION OF REPRODUCTIVE EFFECTS OF PCBs

(ONLY STUDIES ADMINISTERING ORAL AND REPEATED DOSES WERE CONSIDERED)

Internal Study ID	Species	Congener	Dose	Units	Reference
RA002	Rat	Kanechlor 400	0, 25, 75, 225, or 675	mg/kg-d	(Kato <i>et al.</i> , 1972)
RA003	Mouse	Clophen A60	0 or 0.025	mg/d	(Orberg and Kihlstrom, 1973)
RA006	Rat	Aroclor 1242	0, 75, or 150	ppm feed	(Jonsson <i>et al.</i> , 1975)
RA008	Rat	Aroclor 1254	0 or 50	mg/kg-d	(Dikshith <i>et al.</i> , 1975)
RA009	Mouse	PCB4	0, 375, or 750	mg/kg-d	(Torok, 1976)
RA011	Monkey	Aroclor 1248	0, 2.5, or 5	ppm feed	(Allen and Barsotti, 1976)
RA012	Rat	Aroclor 1254	0 or 6.4	mg/kg-d	(Baker <i>et al.</i> , 1977)
RA013	Mouse	Aroclor 1254	0, 25, or 100 (ad libitum); 36, 143 (70% ad libitum)	ppm feed	(Sanders and Kirkpatrick, 1977)
RA015	Rat	Aroclor 1221, 1242, or 1260	0 or 30	mg/kg-d	(Gellert and Wilson, 1979)
RA017	Monkey	Aroclor 1248	0, 2.5, or 5	ppm feed	(Allen <i>et al.</i> , 1980)
RA019	Mouse	PCB169	0, 0.1, 1, 2, 4, 8, or 16	mg/kg-d	(Marks <i>et al.</i> , 1981)
RA021	Monkey	Aroclor 1254	0, 0.1, or 0.4	mg/kg-d	(Truelove <i>et al.</i> , 1982)

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Internal Study ID	Species	Congener	Dose	Units	Reference
RA022	Rat	Aroclor 1254	0, 8, 32, or 64	mg/kg-d	(Sager, 1983)
RA023	Mouse	Aroclor 1254	0, 1.169, 116.69, or 291.69	mg/kg-d	(Talcott and Koller, 1983)
RA024	Rat	Aroclor 1254	0 or 10	mg/kg-d	(Brezner <i>et al.</i> , 1984)
RA025	Monkey	Aroclor 1016	0, 4.5, or 18.1	mg/kg-d	(Barsotti and Van Miller, 1984)
RA026	Mouse	PCB156	0, 10, or 20	mg/kg-d	(Birnbaum <i>et al.</i> , 1985)
RA027	Monkey	PCB77	0, 0.630, or 3.150	mg/kg-d	(McNulty, 1985)
RA028	Mouse	Aroclor 1254	0, 1, 10, or 100	ppm feed	(Welsch, 1985)
RA031	Rat	Aroclor 1254	0.02 (control), 2.5, 26, or 269	ppm feed	(Overmann <i>et al.</i> , 1987)
RA035	Mouse	PCB77	0, 1, 2, 4, 8, 16, 32, or 64	mg/kg-d	(Marks <i>et al.</i> , 1989)
RA036	Monkey	Aroclor 1254	0 or 0.28	mg/kg-d	(Arnold <i>et al.</i> , 1990)
RA037	Monkey	Aroclor 1254	0, 0.005, 0.02, 0.04, or 0.08	mg/kg-d	(Truelove <i>et al.</i> , 1990)
RA041	Rat	Aroclor 1254	0, 0.1, 1, 10, or 25	mg/kg-d	(Gray <i>et al.</i> , 1993)
RA042	Monkey	Aroclor 1254	0, 0.005, 0.02, 0.04, or 0.08	mg/kg-d	(Arnold <i>et al.</i> , 1993a)
RA044	Rat	Aroclor 1254	0, 8, 32, or 64	mg/kg-d	(Sager and Girard, 1994)
RA047	Mouse	Aroclor 1254	0 or 5	mg PCB/kg feed	(McCoy <i>et al.</i> , 1995)
RA048	Monkey	Aroclor 1254	0, 0.005, 0.02, 0.04, or 0.08	mg/kg-d	(Arnold <i>et al.</i> , 1995)
RA051	Monkey	Aroclor 1254	0, 0.005, 0.02, 0.04, or 0.08	mg/kg-d	(Arnold <i>et al.</i> , 1996)

Internal Study ID	Species	Congener	Dose	Units	Reference
RA053	Rat	PCB77 or PCB28	0, 50, 500, 5000, or 50000 (P28); 0, 10, 100, 1000, 10000 (P77)	ppb	(Desaulniers <i>et al.</i> , 1997)
RA060	Mouse	Aroclor 1242	0, 10, 25, 50, or 100	mg/kg-2d	(Fielden <i>et al.</i> , 2001)
RA062	Rat	Mixture (P28, P77, P101, P105, P118, P126, P138, P146, P153, P156, P169, P170, P180, P187)	0, 0.5, or 2, 4	mg/kg-d	(Kaya <i>et al.</i> , 2002)
RA066	Monkey	Aroclor 1242	0 or 0.2	mg/kg-d	(Ahmad <i>et al.</i> , 2003)
RA068	Rat	Aroclor 1254	0 or 25	mg/kg-d	(Meerts <i>et al.</i> , 2004)
RA072	Rat	PCB169	0, 0.03	mg/kg-d	(Yamamoto <i>et al.</i> , 2005)
RA080	Rat	PCB126	0, 0.001, or 0.003	mg/kg-d	(Shirota <i>et al.</i> , 2006)
RA081	Mouse	Mixture (Aroclor 1242, Aroclor 1254)	0, 2.64, or 6.19	mg/kg-d	(Voltura and French, 2007)
RA087	Rat	PCB153	0, 16, or 64	mg/kg-d	(Kobayashi <i>et al.</i> , 2008)
RA089	Rat	PCB153	0, 1, or 4	mg/kg-d	(Kobayashi <i>et al.</i> , 2009)
RA091	Rat	PCB126	0, 0.00001, 0.00003, 0.0001, 0.000175, 0.0003, 0.000550, or 0.001	mg/kg-d	(Yoshizawa <i>et al.</i> , 2009)
RA096	Mouse	Aroclor 1254	0, 0.0005, 0.005, 0.05, or 0.5	mg/kg-d	(Cai <i>et al.</i> , 2011)
RA097	Mouse	Mixture (PCB101, PCB118)	0, 0.001, 0.01, or 0.1	mg/kg-d	(Pocar <i>et al.</i> , 2012)

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Internal Study ID	Species	Congener	Dose	Units	Reference
RA100	Mouse	Mixture (P28, P52, P101, P118, P138, P153, P180)	0 or 0.7	mg/kg-d	(Tomza-Marciniak <i>et al.</i> , 2014)
RA106	Mouse	Aroclor 1254	0, 0.005, or 0.5	mg/kg-d	(Cai <i>et al.</i> , 2015)
RA108	Mouse	Mixture (PCB101, PCB118)	0 or 0.001	mg/kg-d	(Fiandanese <i>et al.</i> , 2016)
RA109	Rat	Aroclor 1254	0, 1, 2, or 5	mg/kg-d	(Sugantha Priya <i>et al.</i> , 2017)
RA113	Rat	Aroclor 1254	0, 1, or 6	mg/kg-d	(Yang <i>et al.</i> , 2009)
RA115	Mouse	Aroclor 1016	0 or 0.05	mg/kg-d	(Gupta, 2000)
RA116	Monkey	Aroclor 1248	0, 0.095, or 0.195	mg/kg-d	(Barsotti <i>et al.</i> , 1976)
RA118	Monkey	Aroclor 1254	0, 0.005, 0.025, or 0.1	mg/kg-d	(Litton Bionetics, 1981)
RA119	Rat	P153	0, 16, or 64	mg/kg-d	(Kobayashi <i>et al.</i> , 2018)
RA120	Rat	Mixture (P77, P126, P169, P105, P118, P138, P153, P180)	0 or 3.96	mg/kg-d	(Hufgard <i>et al.</i> , 2019)
RA121	Rat	A1254	0, 1, 2, or 5	mg/kg-d	(Priya <i>et al.</i> , 2018)
RA122	Rat	P126	0, 0.02, 0.04	mg/kg-d	(Ahmed <i>et al.</i> , 2018)
RA123	Rat	P52	0 or 1	mg/kg-d	(Xie <i>et al.</i> , 2019)